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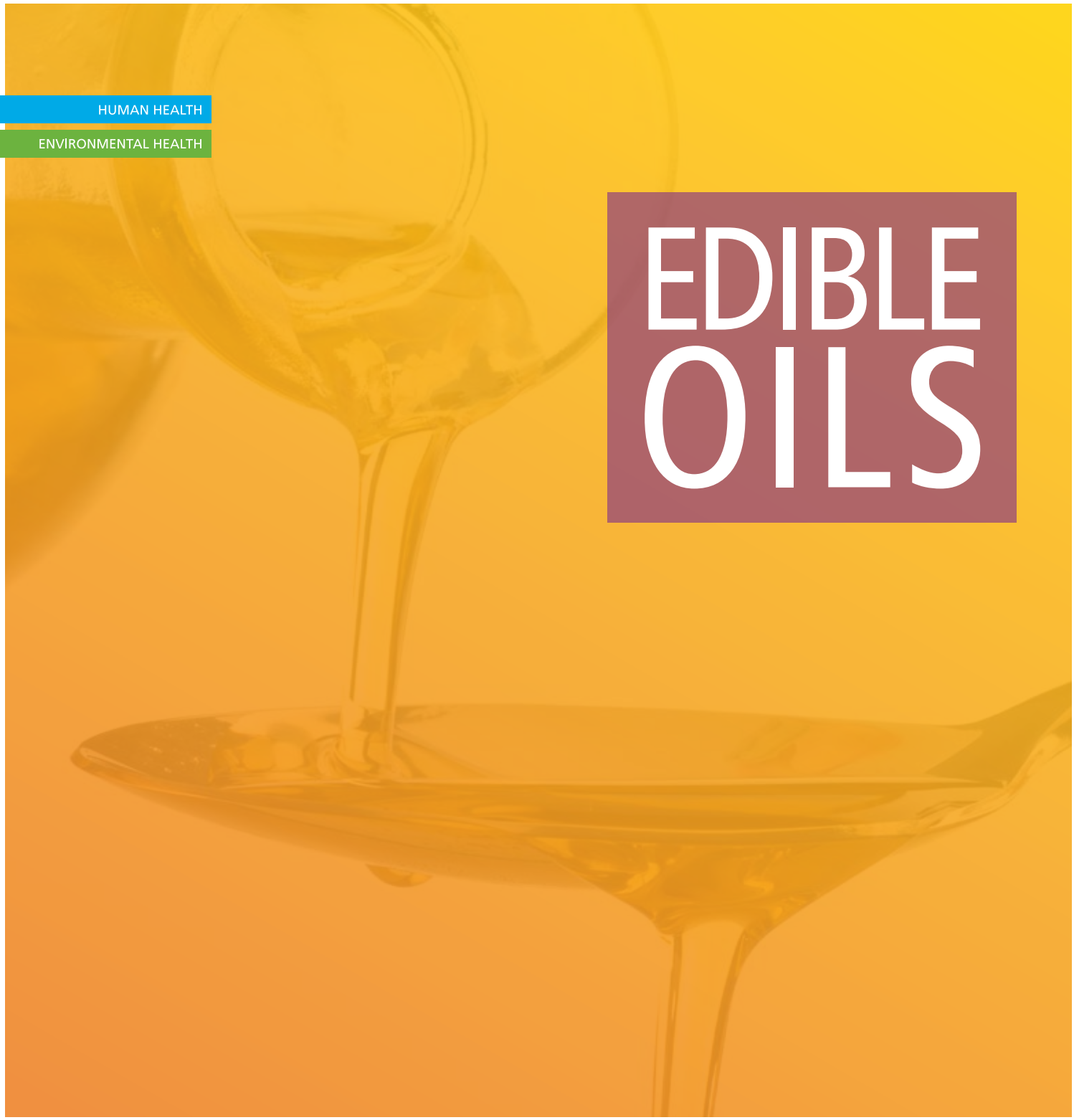

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APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Differentiation of Cultivars of Spanish Olive Oils Using Multiple LC/MS Analysis Methods

Introduction

For high value olive oil, the provenance may have a dramatic effect on the price. Falsifying the origin of oils is financially an attractive proposition to fraudsters. The patterns of organic components in olive oils relate to the geographic origin, cultivar and processing methods for the olives. In this study a statistical analysis of the chemical components of authentic monovariatal virgin olive oils from Spain was used to determine markers for the cultivars. These markers could be used to confirm the provenance and origins of olive oil samples.

Different LC/MS methods were used to measure the levels of a large number of polar and non-polar organic components in the oils. Results from the separate analyses were combined with data fusion. A statistical comparison with the combined results improved the discrimination of cultivar groups, when compared to a single LC/MS analytical method.

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Method

The Spanish Olive Oil Samples

Olive oils produced from a number of olive cultivars grown in Spain were analyzed. Each brand of extra virgin olive oil (EVOO) was pressed from locally grown olives comprised by a single cultivar or blend. In most cases, each cultivar was limited to one distinct geographical region. Olive brands from one cultivar came from two distinct regions.

Oils were produced from either a single olive cultivar of Manzanilla Cacereña, Arbequina, Picual, Arbequina, Empeltre and Cornicabra olives or various blends of Hojiblanco, Picudo and Picual cultivars.

Analytical Methods

The concept of Principal Component Analysis and the implementation of this function within TIBCO Spotfire® software has been described previously (see list of references). A method to extract intensities for each significant feature from LC/MS datasets, resulting in a table with each feature labeled with the mass and elution time of the peaks in the datasets has also been described. The intensities in the table are used as variables for PCA within TIBCO Spotfire®.

Three different sample preparations and LC/MS methods for the analysis of various categories of compounds from olive oil were described in previous application notes relating to olive oil analysis (see list of references). Compounds detected in the oils included triacylglycerides (TAGs), secoiridoids, lignans and fatty acids.

Considerable variation was observed in the levels of these compounds between different brands from the same cultivar, related to different growing conditions, production methods and storable conditions for the oils.

Due to these variations, PCA was unable to separate all the oils from each cultivar into distinct groups using results from any one of the analytical methods described above.

Combining Separate Results Tables with Data Fusion

A data fusion method was explored as a way to improve the discrimination between these chemically very similar oil sample groups. Three separate data tables of LC/MS-derived compound intensities were combined into one fused table for all the detected compounds for each sample. The new fused table enabled a single PCA with all these intensities.

Since the mass spectrometer response varies by compound type, LC conditions, electrospray source parameters and ionization mode; intensities from each separate analysis method have different ranges. Intensities in each table were normalized to unity before the tables were combined.

Grouping Spanish Olive Oils by Cultivar with Data Fusion

The Scores plot (Figure 1, left panel) from PCA of the fused data table produced a clearer grouping of the samples into cultivar groups than was obtained from any single analytical method (see list of references).

For visual clarity, samples from the blended oils were excluded from display in the Scores Plot, although these samples were included in the PCA. The blends clustered in the center of the Scores Plot.

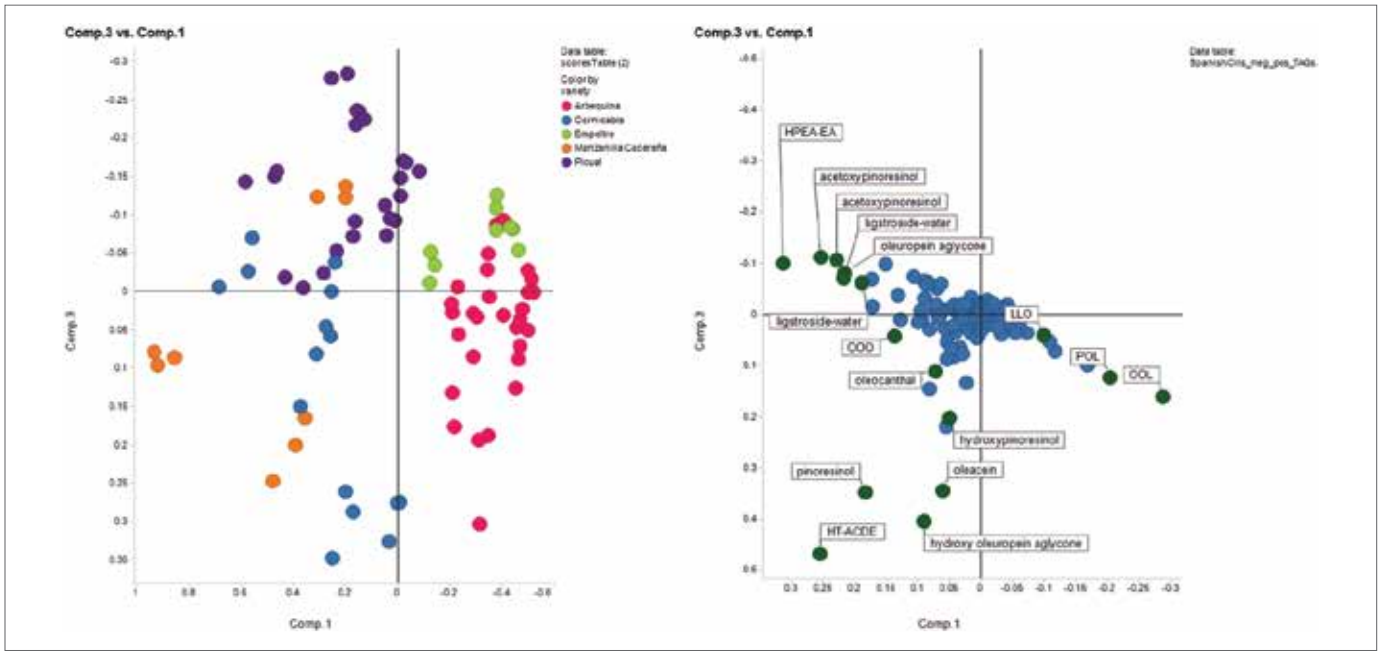

Figure 1. The PC 1 v. 3 Scores and Loadings plots from the PCA of fused data from TAGs and polar compounds detected in negative and positive modes. In the Scores Plot samples are color-coded by cultivar. The plot shows a discrimination of Arbequina and Empeltre cultivars in green and red from those of other olive cultivars.

A distinct separation of oils from Arbequina and Empeltre cultivars from those of the Picual, Cornicabra and Manzanilla Cacereña cultivars was observed, except for one brand of Manzanilla Cacereña oil which did not group with others of that cultivar.

Groups were most clearly differentiated by the variables indicated in the Loadings Plot. Arbequina and Empeltre cultivars had higher levels of the TAGs OOL and OLP and lower levels of many polar compounds, when compared with other cultivars. Picual cultivars had the highest levels of oleuropein aglycone, acetoxypinoresinol and HPEA-EA, or ligstroside aglycone. Cornicabra and Manzanilla Cacereña cultivars had high levels of a number of compounds including pinoresinol, oleacein and HT-ACDE, a hydrotyrosol enolate ester.

Geographic Origins

Brands of Arbequina cultivar oil from two different regions of Spain could be partly separated in a PCA Scores plot due to difference in the levels of a number of TAGs (Figure 2). The level of polar components was not significantly different between oils from the different regions.

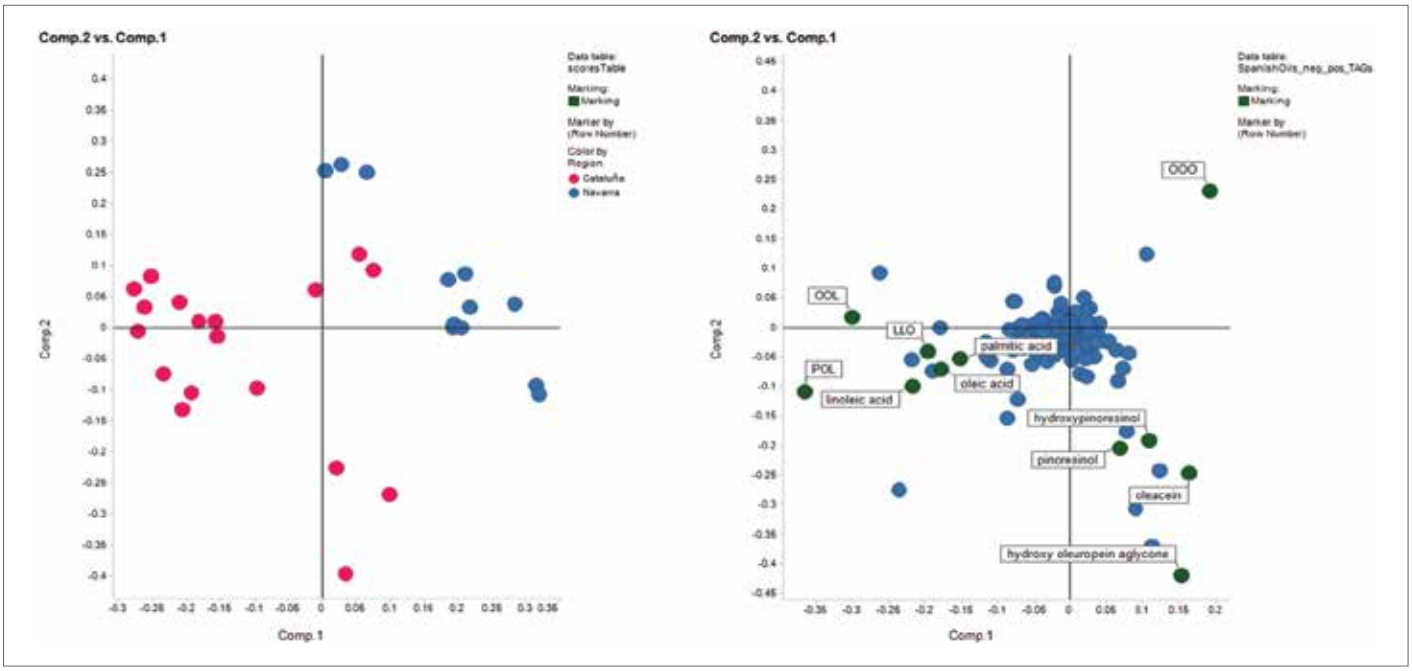

Figure 2. PCA Scores and Loadings plots calculated with the fused information for the Arbequina cultivar samples from two different regions. Partial separation into groups is primarily due to differences in the levels of a number of TAGs.

Conclusion

Data fusion of results from different sample preparation and LC/MS analytical methods allowed for distinct cultivar groups in a PCA Scores Plot. An improved discrimination was produced when compared with PCA using any single LC/MS method.

This concept of fusing together results from separate analytical methods could be extended to include other analytical techniques such as UV and infrared spectral measurements and intensities of compounds from GC/MS analysis and of elements from ICP-MS analysis.

References

1. Differentiation of Olive Varieties by the Triacylglyceride Levels of Oils – Statistical Analysis of LC/MS Results, Application Note, Robert Seward and Catherine Stacey, PerkinElmer, Inc.
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3. Differentiation of Olive, Vegetable and Seed Oils by LC/MS Analysis of Triacylglycerides, Application Note, Robert Seward and Catherine Stacey, PerkinElmer, Inc.

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APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Markers for Spanish Olive Oil Cultivars – Statistical Analysis of Polar Compounds from LC/MS Results

Introduction

For high value olive oil, the country of origin and type of olive cultivar has a dramatic effect on the price. Falsifying the provenance of such oils is an attractive proposition to fraudulent suppliers.

Polar components such as phenolic acids and lignans are present in olive oils.[1] Secoiridoids; phenolics which include an elenolic acid moiety, are unique to the *Olearaceae* family. These compounds are known to have beneficial health effects due to their antioxidant properties and are associated with the organoleptic properties. Measuring the levels of these polar components in oils from different cultivars and geographic origins is of interest for nutritional and authenticity reasons.

In this study a statistical analysis of the polar chemical components of olive oils was used to determine potential markers for olive cultivars. These markers could be used to confirm the provenance of unknown samples.

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Method

Spanish Olive Oil Samples

A number of authentic branded Spanish Extra Virgin Olive oils (EVOOs) were analyzed. Each of the oils were cold pressed from either a single cultivar olive or from a known blend of cultivars grown in a distinct geographic region in Spain. The single cultivars were Manzanilla Cacereña, Arbequina, Picual, Arbequina, Empeltre and Cornicabra and the blends were comprised of various mixtures of Hojiblanco, Picudo and Picual cultivars.

LC/MS Analysis of Polar Components in Olive Oils

Polar Compound Extraction

Liquid-liquid extraction was performed to enrich the polar components of the oils for improved sensitivity. Extraction also reduced the high levels of hydrophobic triacylglycerides and diacylglycerides, which would be retained on reversed-phase columns during the HPLC separation of the polar components.

Olive oil samples were extracted three times with N,N-dimethylformamide (DMF). Extracts were pooled and washed twice with hexane to remove hydrophobic components. Residual hexane was removed from the DMF extracts by centrifugal evaporation. The DMF extracts were diluted 1:20 with water and 2 µL of each extract were injected for LC/MS analysis.

LC/MS Method

Extracts were analyzed in triplicate with a single chromatographic method, with separate analyzes for the detection of components either with positive or negative mode electrospray ionization.

Components were separated by reversed-phase gradients on a Brownlee SPP C18 column with a Flexar™ FX-10 UHPLC pump, using water/methanol eluents at 0.4 mL/min, with a gradient from 10% methanol to 50% methanol over 10 mins, then detected with an AxION® 2 TOF MS fitted with an Ultraspray™ 2 ion source.

An example separation of components for one sample is shown, with the same gradient used for both ionization modes (Figure 1 and Figure 2). Many compounds were selectively detected in either positive or negative modes, although some compounds were detected in both modes. In negative mode (Figure 1), long chain fatty acids and a number of phenolics are detected. In positive mode (Figure 2) a number of phenolics, terpenes and fatty acids are observed as $[M + H]^+$ or $[M + Na]^+$ ions.

For components of interest, the accurate mass and isotope patterns of the molecular ions in the original datasets were used to obtain elemental formulas, which were correlated to known compounds in olive oil. The names and elemental formulas of all the compounds detected in both modes are summarized in a table (see Table 1).

Statistical Analysis

All of the LC/MS datasets were processed with a proprietary algorithm to extract the intensities for significant LC peaks. The results were compiled into a table, with a row for each dataset, and columns of intensities for each significant peak. Each column was labeled with a text summary of the rounded m/z and time values for that peak. The table was imported into TIBCO Spotfire® for statistical analysis using an S-Plus Principal Component Analysis (PCA) function and for graphical display of result in color-coded scatter plots and bar charts.

Methods for extracting the significant features from each LC/MS dataset and for the PCA of these features with Spotfire have been described in previous applications notes.

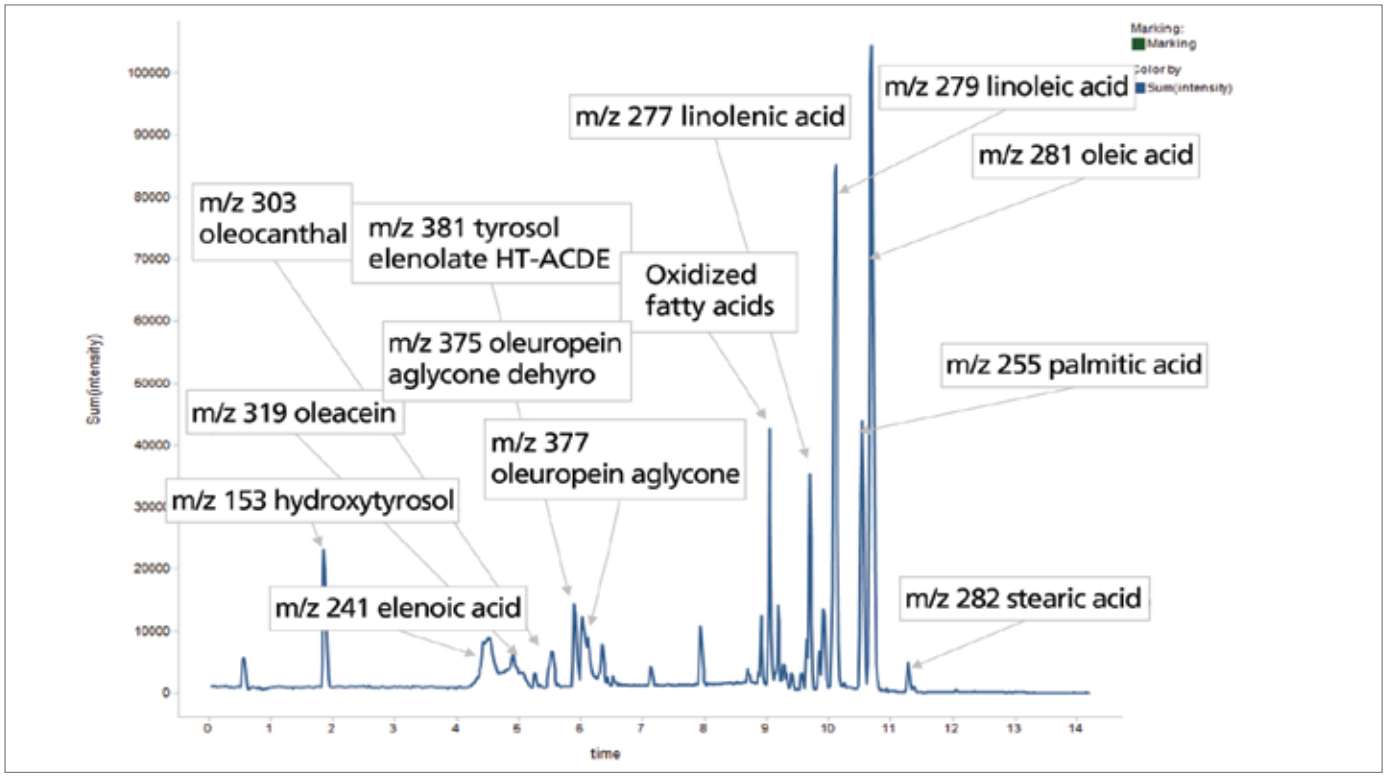

Figure 1. Example separation showing EVOO polar components detected in negative ion mode, with peaks labeled with the nominal mass of each compound.

Negative Mode Statistical Results

The PCA of negative mode features for all the samples gave component scores and loadings which were displayed in scatter plots. A Scores Plot of PC 2 v. 1 (Figure 3 left panel) shows a clear cultivar grouping, with Arbequina and Empeltre samples grouped in the left side of the plot and are distinguished from Cornicabra, Picual and blends on the right side.

The components which most strongly contribute to the cultivar differences are shown in the corresponding Loadings Plot (Figure 3 right panel). Significant differentiators of the groups include the levels of oleic acid, together with the phenolics hydroxytyrosyl acyclodihydroelenolate (HT-ACDE) and elenolic acid.

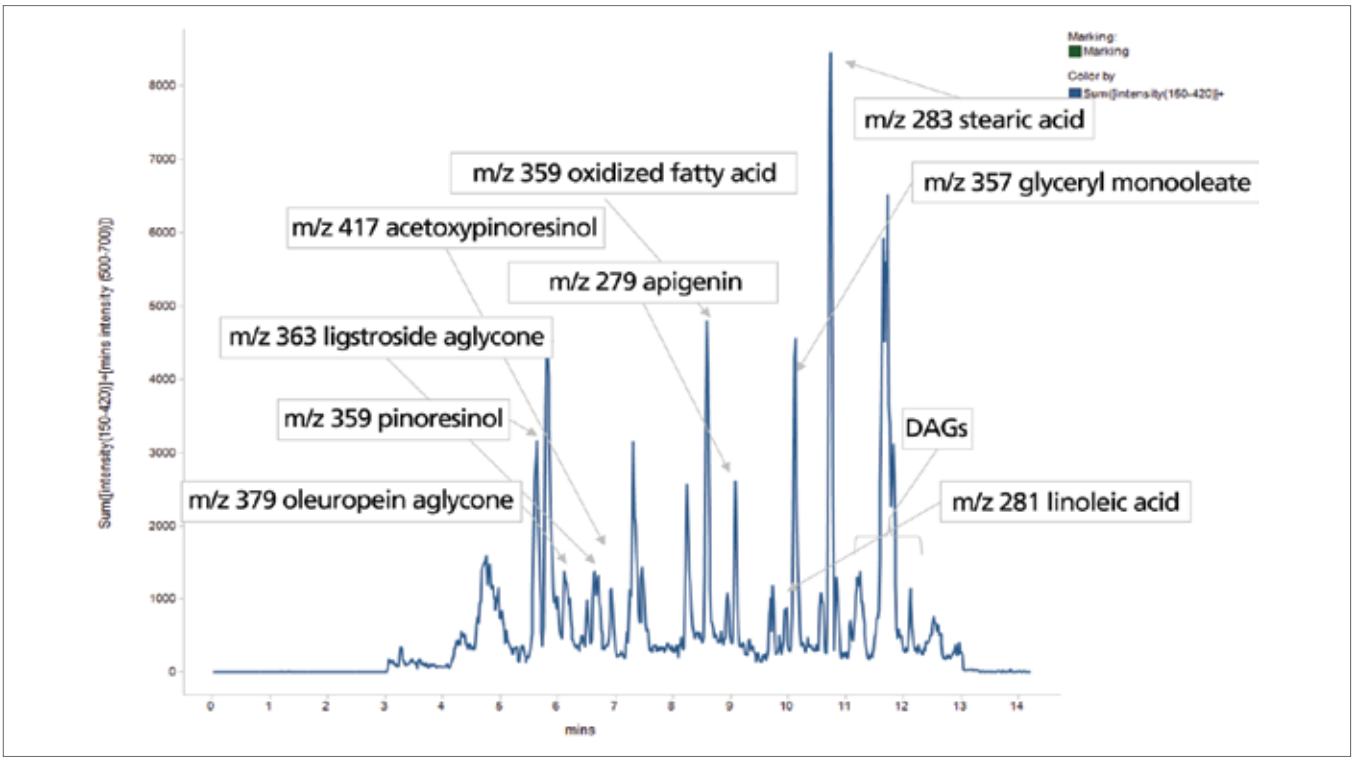

Figure 2. Example separation showing EVOO polar components detected in positive ion mode, with peaks labeled with the nominal mass of each compound.

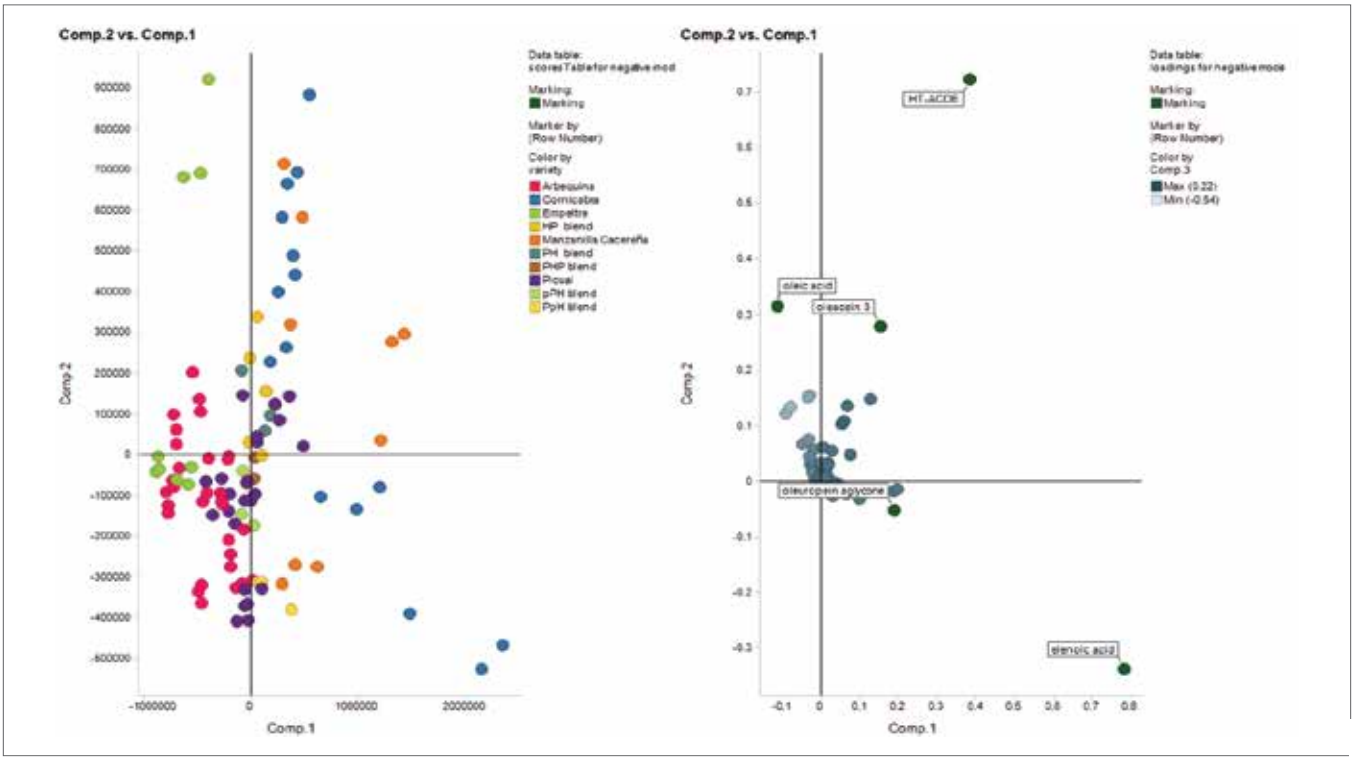

Figure 3. PCA Scores Plots and Loadings Plots from the negative mode results for all detected compounds. Grouping of samples by cultivar (left panel) most strongly correlates to the levels of the compounds HT-ACDE and elenolic acid as shown in the Loadings Plot (right panel).

All cultivars had similar levels of fatty acids, except for one Empeltre cultivar which had higher levels. Normally free fatty acid levels in EVOOS are low, but high levels are related to the breakdown of TAGs in the olive oil. The high levels in this one sample suggest that the oil may be degraded. These results suggest that levels of free fatty acids are not reliable markers for cultivar or origin.

The levels of phenolics vary widely between cultivars and between different brands within each cultivar. Some brands have extremely high levels of elenoic acid. Because of a chemical transformation of elenolic acid in acidic eluents, a broad peak was observed with the chromatography method used. Thus, the reported peak height and area were not as reproducible between samples as for other compounds, leading to a wide scatter of sample points in the initial Scores Plot.

A new PCA of the negative mode results excluded the elenolic acid and fatty acids component intensities which had shown large variability in the first analysis. The new Scores Plots (Figure 4 left panel) now show groupings due only to the variation in levels of the phenolic compounds. Arbequina, Empeltre and Picual samples are each closely grouped, although Cornicabra and Manzanilla Cacereña samples were scattered.

The compounds in the Loadings Plot (Figure 4 right panel) which were most related to the cultivar grouping were oleuropein aglycone, oleocein, HT-ACDE and ligstroside aglycone decarboxymethyl (p-HPEA-EDA or oleocanthal).

Two of these compounds, oleuropein and oleocanthal, contribute to the bitterness of the oils. Levels are lowest in the Arbequina and Empeltre cultivars, which have low bitterness levels, as shown in a bar chart (Figure 5).

Variability of oleuropein levels for brands within each cultivar may be due to different ripeness levels of the olives used for the oils. Oleuropein levels are reduced during the ripening of the olives. A recent report[1] analyzed the levels of oleocanthal and oleacein in Greek and Californian oils and found levels varied by both cultivar and processing temperature.

Positive Mode Statistical Results

The PCA of positive mode phenolic compounds levels produced a Scores Plot of PC 2 v. 1 (Figure 6, left panel), which showed cultivar grouping. The Loadings Plot showed that the compounds ligstroside aglycone (p-HPEA-EA or oleocanthal), oleuropein aglycone (DHPEA-EA), acetoxypinoresinol and pinoresinol were most correlated to the cultivars.

The lignans pinoresinol and acetoxypinoresinol showed widely varying levels between cultivars. All Manzanilla Cacereña, some Cornicabra and Picual cultivars and blended samples have high levels of both lignans; Empeltre and Arbequina brands had no detectable acetoxypinoresinol and moderate levels of pinoresinol.

These lignans have been detected previously[2,3] in Spanish olive oils. Levels of acetoxy-pinoresinol were reported to be abundant in Arbequina and Hojiblanco oils, but low in Picual and Hojiblanco oils; with pinoresinol abundant in Picual and Cornicabra cultivars.

Our findings differ, but a large variation in levels of these lignans between oils from different brands was predictable. Pinoresinols are major components of olive seeds, so levels may be related to the different olive crushing conditions rather than cultivar.

Free fatty acids are also detected in positive mode as $[M + H]^+$ ions. Oleic acid has high levels in one Empeltre cultivar, similar to the findings from negative ion results. 2-oleoylglycerol, resulting from oxidative degradation of TAGs, was also detected in positive mode and has highest levels in the same Empeltre cultivar sample. Other compounds proposed to be oxidized forms of oleic acid are also higher in all Empeltre samples.

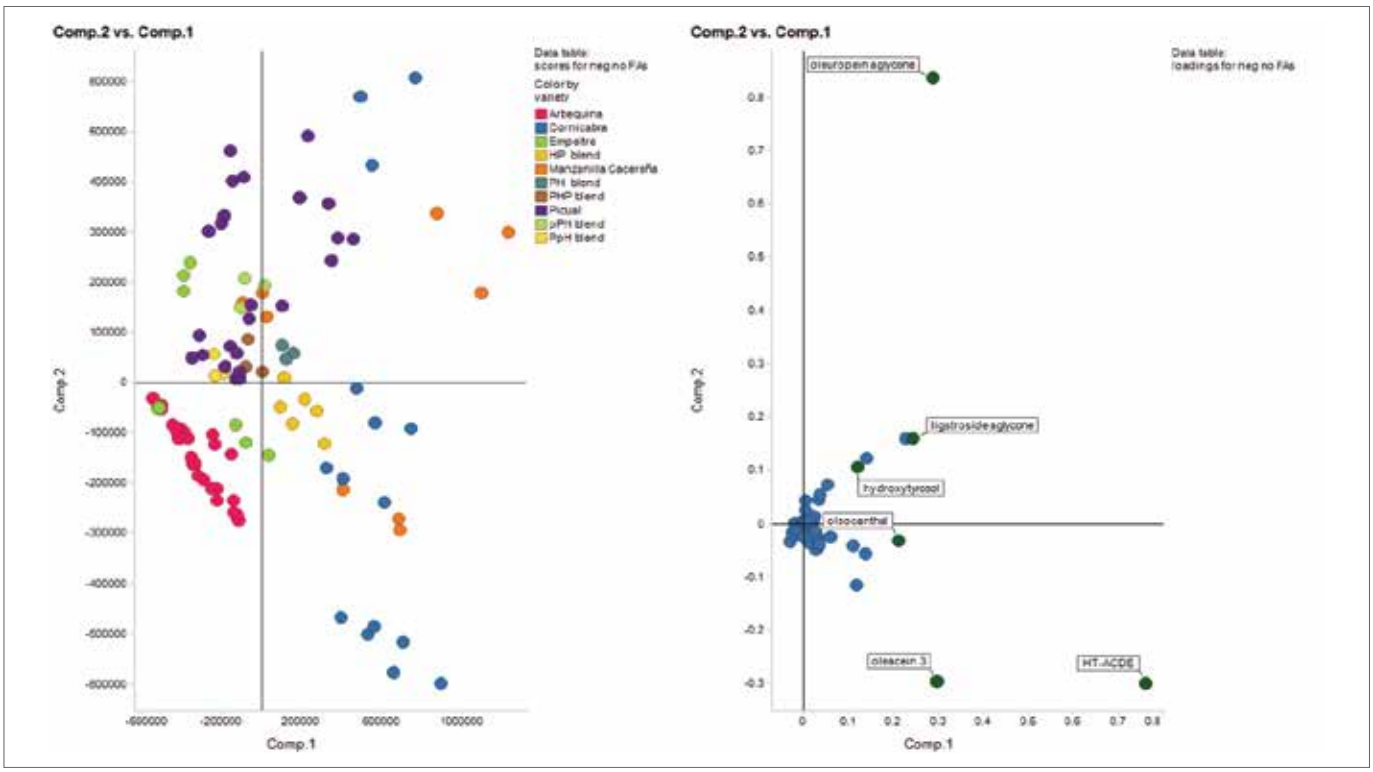

Figure 4. PCA Scores Plots and Loadings Plots of phenolics detected in negative mode. Scores Plot (left panel) shows grouping correlated to levels of oleuropein aglycone, oleocein, HT-ACDE and ligstroside aglycone (p-HPEA-EDA).

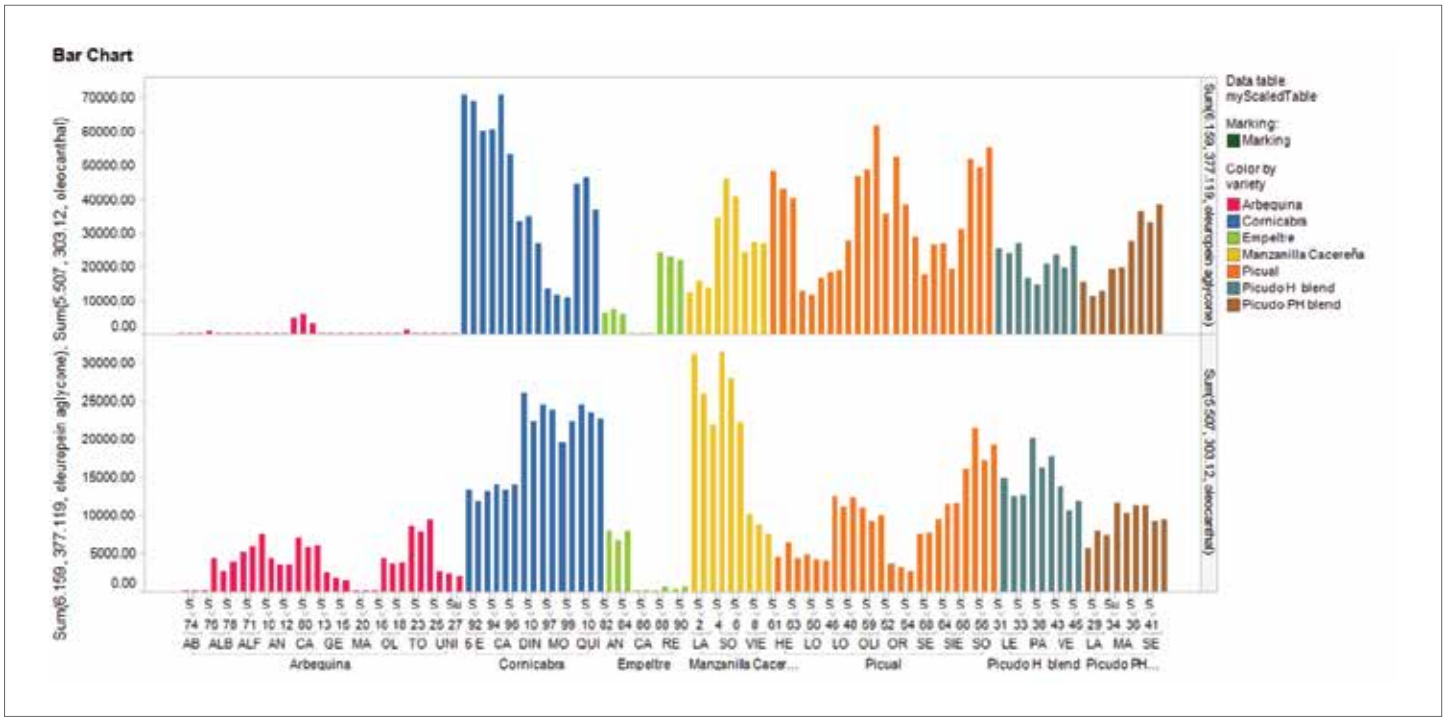

Figure 5. Levels of the bitter compounds oleuropein (top) and oleocanthal (bottom) for each sample, ordered and color coded by cultivar. Levels of both are lowest in the Arbequina (red) and Empeltre (green) cultivars.

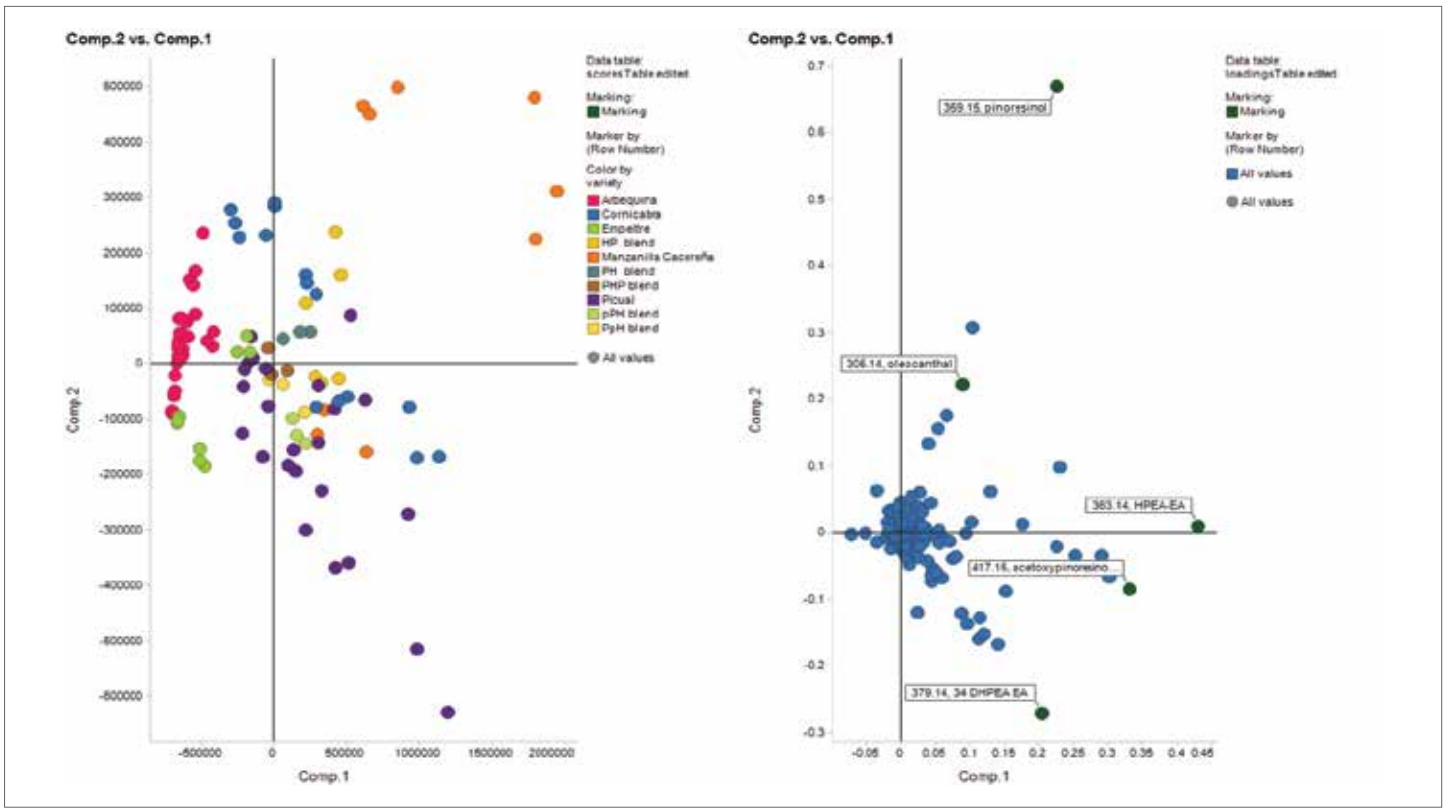

Figure 6. PCA Scores and Loadings Plots of the positive mode results. Scores Plot (left panel) shows grouping into cultivars dominated by levels of acetoxypinoresinol, pinoresinol, ligstroside aglycone HPEA-EA and oleuropein aglycone DHPEA-EA.

Table 1. Accurate masses of the compounds.

Molecule	[M-H]-	[M+H]+	[M+Na]+
Tyrosol (p-HPEA)	137.0608	139.0754	161.0578
Hydroxytyrosol (3,4-DHPEA)	153.0557	155.0703	177.0528
Hydroxytyrosol aetate (3,4-DHPEA-AC)	195.0663	197.0808	219.0633
Hydroxylated decarboxymethyl elenolic acid	199.0612	201.0757	223.0582
Hydroxylated decarboxyl elenolic acid	213.0768	215.0914	237.0739
Desoxy elenolic acid	225.0768	227.0914	249.0739
Myristic acid	227.2017	229.2162	251.1987
Elenolic acid (EA)	241.0718	243.0863	265.0688
Palmitoleic acid (16:1)	253.2173	255.2319	277.2143
Elenolic acid methyl ester	255.0874	257.1020	279.0845
Palmitic acid (16:0)	255.2330	257.2475	279.2300
Hydroxylated elenolic acid	257.0667	259.0812	281.0637
Apigenin	269.0455	271.0601	293.0426
Linolenic acid (18:3)	277.2173	279.2319	301.2143
Linoleic acid (18:2)	279.2330	281.2475	303.2300
Oleic acid (18:1)	281.2486	283.2632	305.2456
Steric acid (18:0)	283.2643	285.2788	307.2613
Luteolin	285.0405	287.0550	309.0375
Tyrosol hexoside	299.1136	301.1282	323.1107
Ligstroside aglycone decarboxymethyl (p-HPEA-EDA, Oleocanthal)	303.1238	305.1384	327.1208
Hydroxytyrosol 4-O-glucoside	315.1085	317.1231	339.1056
Oleuropein aglycone decarboxymethyl (3,4-DHPEA-EDA, Oleacein)	319.1187	321.1333	343.1158
Lactone (ester with hydroxytyrosol)	321.1344	323.1489	345.1314
10-OH-Oleuropein aglycone decarboxymethyl	335.1136	337.1282	359.1107
Pinoresinol	357.1344	359.1489	381.1314
Ligstroside aglycone dehydro	359.1136	361.1282	383.1107
Ligstroside aglycone (p-HPEA-EA)	361.1293	363.1438	385.1263
(+)-1-Hydroxypinoresinol	373.1293	375.1438	397.1263
Oleuropein aglycone dehydro	375.1085	377.1231	399.1056
Oleuropein aglycone (3,4-DHPEA-EA)	377.1242	379.1387	401.1212
Hydroxytyrosol acyclodihydroelenolate (HT-ACDE)	381.1555	383.1700	405.1525
Oleoside / Secologanoside	389.1089	391.1235	413.1060
Methyl oleuropein aglycone	391.1398	393.1544	415.1369
Hydroxy-oleuropein aglycone	393.1191	395.1337	417.1162
Oleoside 11-methylester / oleoside 7-methyl ester / 8-epikingiside	403.1246	405.1391	427.1216
(+)-1-Acetoxypinoresinol	415.1398	417.1544	439.1369
Oleoside dimethylester	417.1402	419.1548	441.1373
Ligstroside aglycone + MeOH + FA	439.1610	441.1755	463.1580
Oleanolic acid	455.3531	457.3676	479.3501
Maslinic acid	471.3480	473.3625	495.3450

Conclusion

Oils from a number of olive cultivars could be partly differentiated by the levels of polar component features detected in either positive or negative mode analysis. The most significant differentiators determined from PCA are certain lignans and secoiridoids, including pinoresinol, acetoxypinoresinol, oleuropein and oleocanthal.

Methods combining these results, from separate analytical methods, for more of a complete statistical evaluation of the oils, will be covered in a separate application note.

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APPLICATION NOTE

Mass Spectrometry

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Rapid Measurement of Olive Oil Adulteration with Seed Oils with Minimal Sample Preparation Using DSA/TOF

Introduction

Olive oil is a valuable product that is traditionally produced in Mediterranean countries and now in the United States (particularly California), and has nutritional advantages concerning cardiovascular disease prevention[1,2]. Due to its high nutritional value, it is offered at a higher price than other seed oils. For this reason, it is sometimes adulterated with other cheaper seed oils.

Olive oil and other oils are composed mainly of triacylglycerols. These molecules are derived from the esterification of three fatty acid molecules with a glycerol molecule. The main triglyceride in olive oil is triolein (OOO); whereas the main triglyceride in seed oils (soybean, corn and sunflower) is trilinolein (LLL). Therefore, the measurement of ratio of trilinolein to triolein in olive oil can be used as a way to detect its adulteration with soybean and other seed oils, such as corn and sunflower, which have a higher content of trilinolein and lower amount of triolein in comparison to olive oil[3]. Using this strategy with a Direct Sample Analysis™ Time-of-Flight mass spectrometry system (DSA/TOF), we detected adulteration of olive oil with soybean and corn oil. In the past, methods employing gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC) hyphenated to MS (HPLC/MS) were implemented for this purpose[4-7]. These methods are time consuming, expensive and require extensive sample preparation, method development and derivatization. In this work, we demonstrated that the AxION® Direct Sample Analysis (DSA™) system integrated with the AxION 2 Time-of-Flight (TOF) mass spectrometer can be used for rapid screening of adulteration of olive oil with seed oils such as soybean and corn oil with minimal sample preparation.

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Experimental

Olive oil, soybean and corn oil were purchased from a local supermarket. All oils were diluted to 1 % in iso-propanol. After dilution, the oils were mixed in different proportions to simulate the adulteration of olive oil with soybean oil and corn oil at different percentages of 5, 10, 25 and 50 %. To obtain excellent mass accuracy, the AxION 2 TOF instrument was calibrated before each analysis by infusing a calibrant solution into the DSA source at 10 µl/min. Five µl of each sample was pipetted directly onto the stainless steel mesh of the AxION DSA system for ionization and analysis. All samples were analyzed within 30 seconds.

The DSA/TOF experimental parameters were as follows:

Mass spectrometer:	PerkinElmer AxION 2 TOF MS
Ionization source:	PerkinElmer AxION Direct Sample Analysis (DSA)
Ionization mode:	Positive
Flight Voltage:	-8000 V
Mass Scan Range:	10-1100 Da
Acquisition Rate:	5 Spectra/s
Capillary exit voltage:	150 V
DSA source temperature:	300 °C
Drying gas flow rate:	4 L/min

Results

Figures 1, 2 and 3 show the mass spectra for a 1 % solution of olive, soybean and corn oil in iso-propanol in positive ion mode using DSA/TOF, respectively. The mass spectra shows that the main triglyceride in olive oil is triolein; whereas the main triglyceride in soybean and corn oil is trilinolein. The data shows that the response ratio for trilinolein to triolein (LLL/OOO) was 0.033, 4.25 and 9.2 in olive oil, soybean oil and corn oil, respectively. Therefore, the higher response ratio for trilinolein to triolein in olive oil can be used to detect adulteration of olive oil with soybean or/and corn oil using DSA/TOF. This is supported further by data in Figure 4 which shows that the response ratio of trilinolein to triolein increased when the addition of soybean oil went from 5 to 50 % in eight different olive oils. Similarly, Figure 5 shows that the response ratio of trilinolein to triolein increased with increase in corn oil adulteration from 5 to 50 % in eight different olive oils.

The extensive data collected in this work shows that average response ratio for trilinolein to triolein in olive oils was 0.033 with standard deviation of 0.013. Therefore, if an olive oil showed the response ratio for LLL/OOO at a value higher than 0.072 (calculated using value of average + 3 times standard deviation for LLL/OOO response ratio in olive oil) with DSA/TOF, it would indicate that it might be adulterated with soybean or corn oil, or any oil containing a higher level of trilinolein than olive oil.

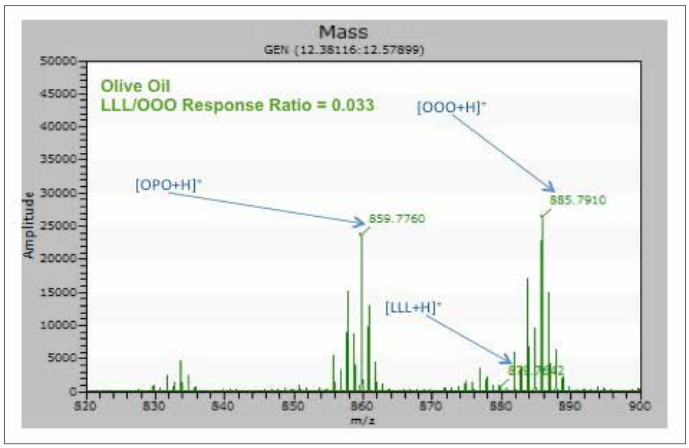

Figure 1. Mass spectra of olive oil in positive ion mode using DSA/TOF.

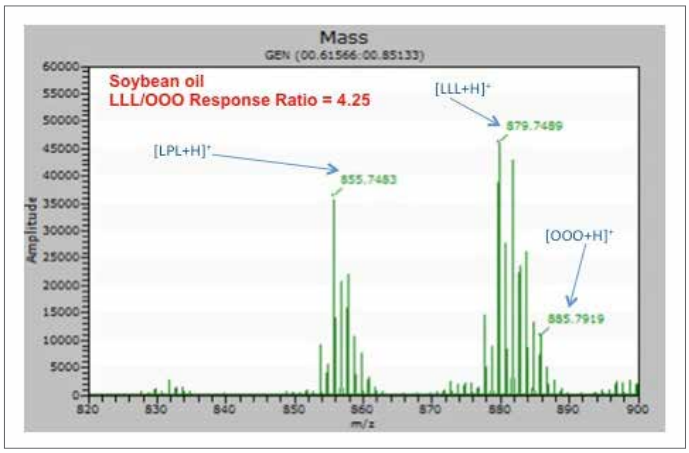

Figure 2. Mass spectra of soybean oil in positive mode using DSA/TOF.

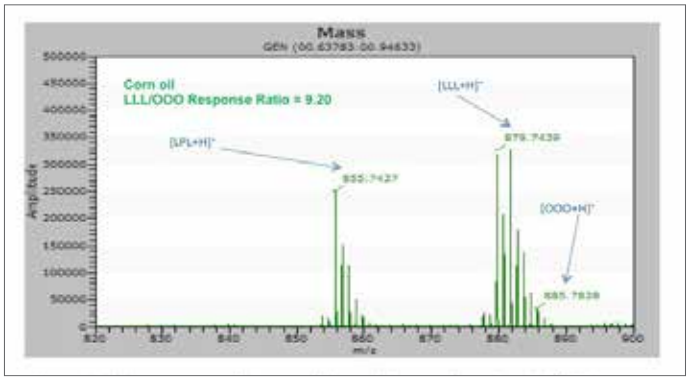

Figure 3. Mass spectra of corn oil in positive mode using DSA/TOF.

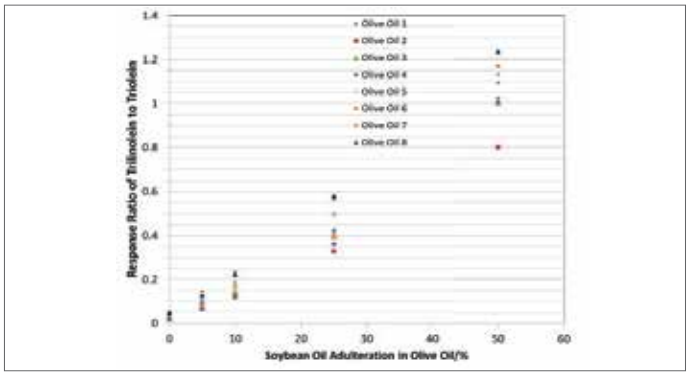

Figure 4. Effect of olive oil adulteration with different levels of soybean oil on response ratio of trilinolein to triolein.

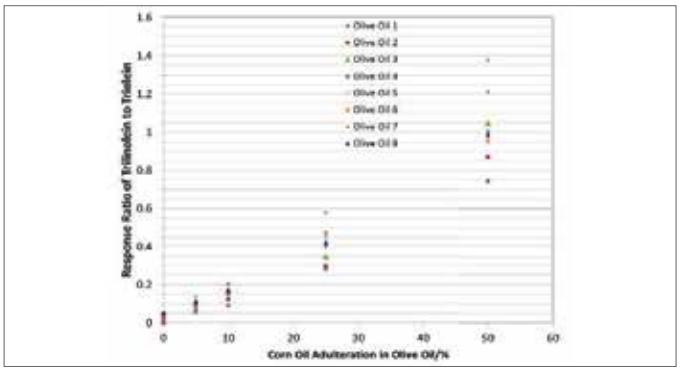

Figure 5. Effect of olive oil adulteration with different levels of corn oil on response ratio of trilinolein to triolein.

Conclusion

This application shows a rapid method for screening olive oil adulteration with other seed oils such as soybean and corn oil using DSA/TOF. The data showed that the higher response ratio for trilinolein to triolein in olive oil can be used to detect its adulteration with soybean or corn oil. All samples were screened with minimal sample preparation and in 30 seconds per sample. In comparison to other established techniques such as LC/MS and GC/MS, DSA/TOF can improve laboratory productivity and decrease operating costs and analysis time.

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APPLICATION NOTE

Near-Infrared Spectroscopy

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Quality Control of Olives by Near-Infrared Spectroscopy and AssureID Software

Introduction

Olive oil is an increasingly popular food product worldwide, with global production exceeding 3.0 million tons in 2011 and showing steady annual growth. Despite these huge volumes, however, margins are relatively small in olive oil production so quick and easy analysis of oil quality is vital to maintain process efficiency. Rapid, reliable analysis can contribute to process and quality improvements in numerous ways. For example,

- Assessment of raw olive acceptability. If the olives have been collected from the ground rather than fresh from the tree, they may be of poor quality with high acidity and hence lower value.
- Measurement of water and oil content. These parameters determine the price of the olives, with those having a greater oil content commanding a higher price.
- Process optimization. After extracting the oil, the remaining pulp or by-product (called alperujo in Spain) should have only minimal oil content, typically around 2% or less. If the oil exceeds this level, a problem with the process is indicated.

This note describes how a PerkinElmer Frontier™ FT-NIR spectrometer and AssureID software have been used by an olive oil producer in Spain to improve productivity by implementing the above analyses within their routine production.

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Materials and Methods

A PerkinElmer Frontier FT-NIR spectrometer equipped with an upwards-facing reflectance accessory and sample spinner (NIRA) was used for all measurements.

Olive samples were milled to a paste and placed in a glass petri dish before analysis. Spectra were collected between 10000 and 4000 cm^{-1} at 16 cm^{-1} resolution, with an accumulation time of 30 seconds per sample.

The olive samples were also analyzed for oil and water content following the customer's established laboratory procedures.

Some of the measured spectra are shown in Figure 1. Typically for NIR spectra, the absorption features are broad and overlapped, although several prominent features can be assigned either to water or to organic C–H modes in the oil.

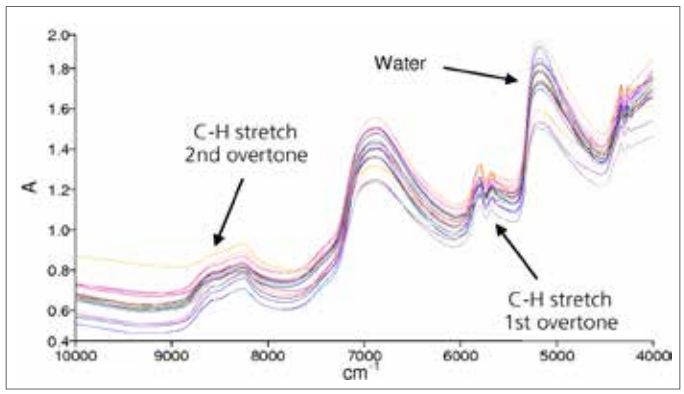

Figure 1. NIR spectra of some of the olive samples.

Assuring Olive Quality

SIMCA is a powerful chemometric method for sample classification that builds independent models for each sample class – in this case, fresh and old olives. New samples are tested against both models, and identified as belonging (or not) to one of the material classes. Compared to traditional methods of identification such as spectral correlation, SIMCA has a much greater ability to distinguish between relatively similar materials, even in the presence of natural variation – provided this is captured in the training set data used to build the models.

AssureID software was designed from the ground up to streamline the process of building SIMCA models, and breaks the procedure down into a series of straightforward, logical steps:

1. Define materials and acquire spectra of known references.
2. Optionally, configure algorithm parameters and spectral pre-processing such as baseline correction: the default settings are tailored to the instrument and sample type and in most cases will produce good results without modification.
3. Calibrate the method. The software automatically builds the models and determines the acceptance thresholds.
4. Review the classification results (for example, see Figure 2). Any issues with the data or performance of the method will be flagged by the troubleshooting engine, allowing corrective action to be taken.
5. The validated method is then deployed as a workflow within the dedicated Analyzer module of AssureID, allowing routine use of the method.

Quantitative Modeling of Oil and Water Content

The oil and water contents of the olives are key parameters for quality, and both contribute to the NIR spectrum. The complex nature of NIR spectra often makes it impossible to develop quantitative models based on the absorbance at a single wavelength. However, multivariate (chemometric) methods such as partial least squares regression (PLS) still function in the presence of overlapping bands, and can allow models to be built.

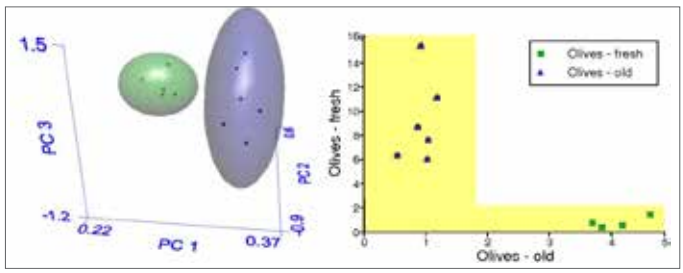

Figure 2. Overview PCA (left) and Cooman's (right) plots for the models to discriminate old and fresh olives. Each axis represents the residual distance against one model. A clear separation of points in the top-left and bottom-right corners, as seen here, indicates that the model is comfortably distinguishing the two types of olive.

The olive spectra and properties determined by chemical analysis were loaded into PerkinElmer Quant+ software. One third of the data were designated as a validation set to verify the performance of the model. The spectra were pre-processed with first-derivative baseline correction.

The calibration and validation results are summarized in Table 1 and Figure 3. The models use a modest number of latent variables and show good linearity and precision over the range of available samples. The standard errors of prediction (SEPs) were 1.5 % and 1.7 % for oil and water, respectively.

Table 1. Summary of results for the PLS modeling of oil and water in olives.

Property	Oil (%)	Water (%)
Range	14–41	34–61
Mean	25	46
Standard deviation	5	6
No. of latent variables	5	3
Validation SEP	1.5	1.7

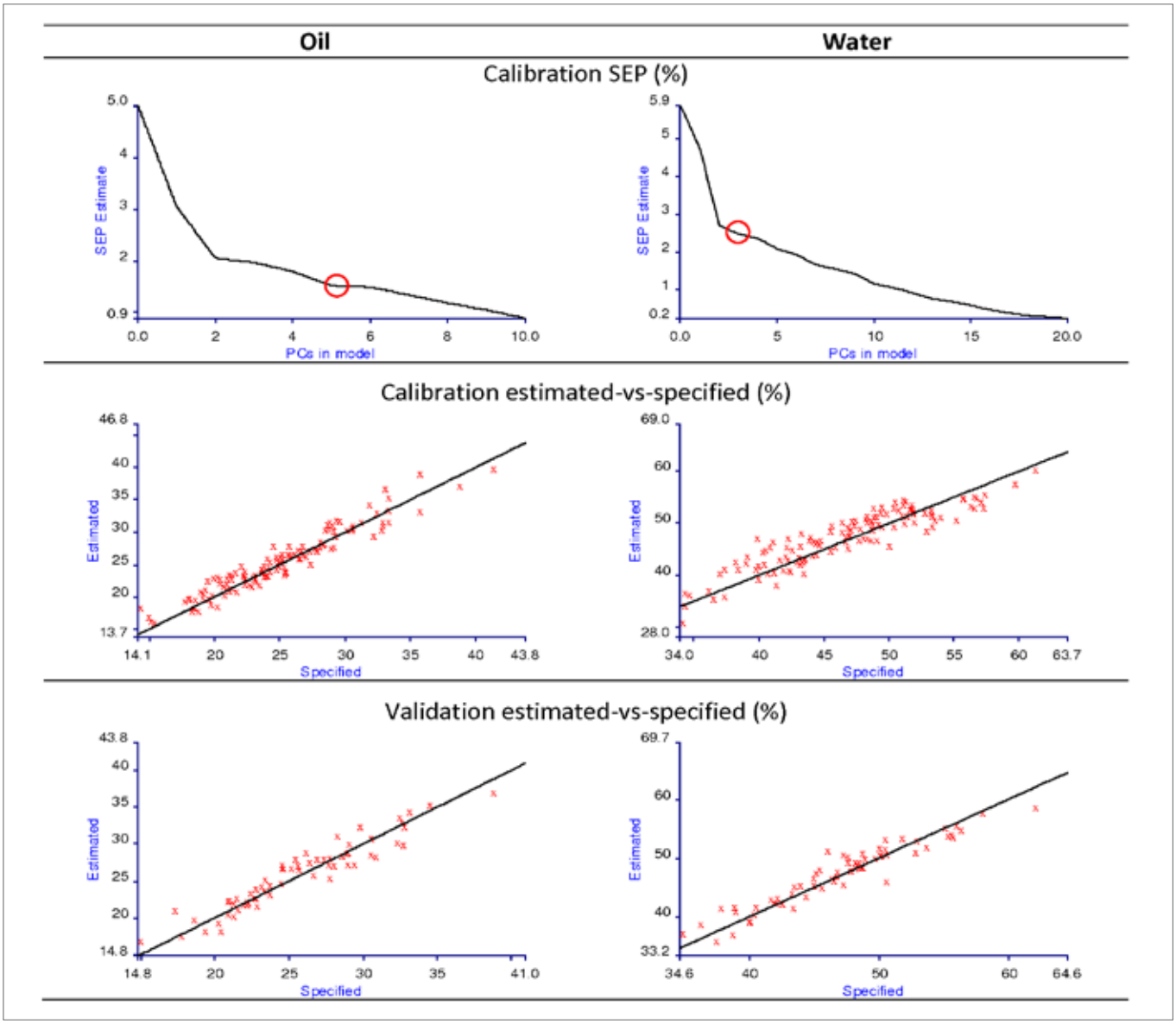

Figure 3. Calibration and validation results for oil (left column) and water (right column).

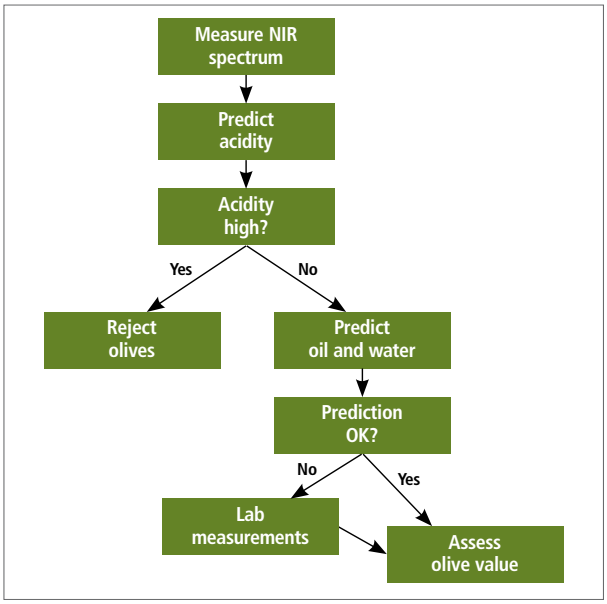

Figure 4. Flow chart for olive analysis by NIR spectroscopy with AssureID.

These quantitative models were also incorporated into the AssureID analysis. After checking the olives for quality, acceptable olives will be further analyzed for oil and water content (as shown in the flowchart in Figure 4).

While AssureID allows sophisticated analyses using both qualitative and quantitative chemometric methods, its design as separate method-building and analysis modules ensures that the end-user is presented with a simple interface, as shown in Figure 5.

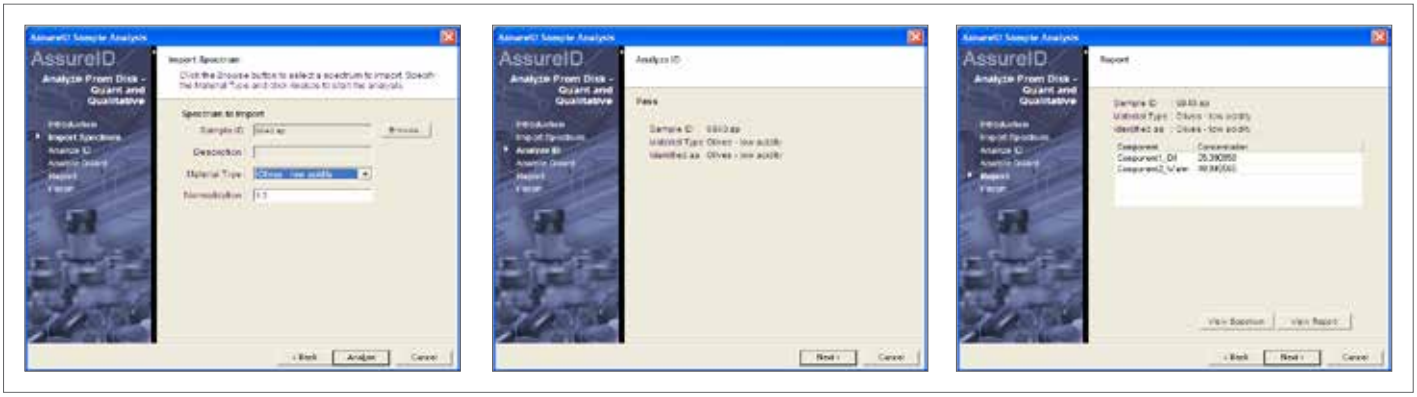

Figure 5. AssureID workflow for olive analysis.

Conclusions

Increasing pressure on food and food ingredient manufacturers to increase efficiency while maintaining product quality has created a need for rapid and precise analysis of materials at all stages of the processing chain. Near-infrared spectroscopy provides rich information about physical and chemical properties of many food materials, and combined with chemometric techniques can provide unequalled speed and precision of analysis. In this note we have shown how the Frontier near-infrared spectrometer from PerkinElmer, in conjunction with AssureID software, is being used to perform three key analyses in olive processing: checking for excess acidity to reject poor-quality olives, measuring the oil and water content to assess olive value, and measuring the oil content in the alperujo by-product to verify extraction efficiency.

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APPLICATION NOTE

UV/Vis Spectroscopy

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Determination of Olive Oil Purity and Degree of Oxidation using the LAMBDA XLS

Introduction

Olive oil is well established in the food industry. Demand continues to grow not only because of its distinct flavor, but also because of an increased awareness of its health benefits. In fact, the FDA allows producers of olive oil to place a health claim on their products because there is some scientific evidence to support a risk reduction of coronary heart disease by consuming a higher proportion of monounsaturated fat in one's diet. This is significant because olive oil is considerably rich in monounsaturated fats, most notably oleic acid. It is therefore of interest to producers to know the quality of the oil, its state of preservation, and changes brought about in it by technological processes.

The quality of the olive oil is studied by measuring the characteristics of the absorption bands between 200 and 300 nm. These are frequencies related to conjugated diene and triene systems. A low absorption in this region is indicative of a high-quality extra virgin olive oil, whereas adulterated/refined oils show a greater level of absorptions in this region.

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Instrumentation

The PerkinElmer LAMBDA™ XLS UV/Vis spectrophotometer, shown in Figure 1, is a standalone, robust scanning spectrophotometer with no moving parts and a unique Xenon® Lamp Source (XLS) with a typical lifetime of five years.

The LAMBDA XLS is equipped with a large LCD screen making running methods and viewing data easier. Results can be printed, stored, or exported into Microsoft® Excel® for use on your personal computer.

Method

Olive oil samples were diluted in iso-octane (2,2,4-trimethylpentane). All samples were measured in matched, synthetic fused silica cuvettes (10 mm is the recommended pathlength) running a solvent blank as a reference.

Absorption measurements for purity determination were made at 232, 266, 270 and 274 nm. K values were calculated according to the equation shown in Figure 2.

The purity of olive oil can be determined from three parameters:

- K232 absorbance at 232 nm
- K270 absorbance at 270 nm
- Delta K (Figure 3)

The LAMBDA XLS was used to collect UV data from four different label graded commercial olive oil samples.

Results

The results collected using the LAMBDA XLS for the four different graded olive oils are shown in Table 1.

Conclusion

The LAMBDA XLS is a reliable and cost effective system appropriate for keeping up with regulations around the standard method for measuring olive oil purity using a UV spectrophotometric technique.

Table 1. Measured and Permitted K Values of Commercial Olive Oils.

	Measured K Values				
Olive Oil Sample Type	K232	K266	K270	K274	Delta K
Extra Virgin - Sample 1	1.897	0.151	0.148	0.135	0.005
Extra Virgin - Sample 2	1.717	0.201	0.189	0.173	0.002
Virgin - Sample 3	1.436	0.240	0.248	0.223	0.016
Olive Oil - Sample 4	3.000	0.640	0.832	0.458	0.283

Figure 1. LAMBDA XLS UV/Vis spectrophotometer.

$$K\lambda = \frac{Abs\lambda}{D \times L}$$

D = Dilution gr/L

L = cuvette pathlength

Figure 2. K equation for λ nm.

$$\text{Delta K} = K_{270} - \frac{K_{266} + K_{274}}{2}$$

Figure 3. Delta K equation.

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APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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The Determination of Low Levels of Benzene, Toluene, Ethylbenzene, Xylenes and Styrene in Olive Oil Using a TurboMatrix HS and a Clarus SQ 8 GC/MS

Introduction

Levels of benzene, toluene, ethylbenzene, xylenes and styrene (BTEXS) are a concern in olive oil. These compounds find their way into olive trees and hence into the olives and olive oil mainly as a result of emissions from vehicles, bonfires, and paints into ambient air near the orchards.

Various methods have been developed to detect and quantify these compounds down to levels of 5 ng/g (5 ppb w/w). This application note describes an easy to perform method using PerkinElmer® Clarus® SQ 8 GC/MS with a TurboMatrix™ 110 headspace sampler to achieve detection limits below 0.5 ng/g.

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Method

The experimental conditions for this analysis are given in Tables 1 to 4.

Table 1. **GC Conditions.**

Gas Chromatograph	**Clarus 680**
Column	30 m x 0.25 mm x 1.0 µm Elite-Wax
Oven	35 °C for 1 min, then 10 °C/min to 130 °C
Injector	Programmable Split Splitless (PSS), 180 °C, Split OFF
Carrier Gas	Helium at 1.0 mL/min constant flow (7.2 psig initial pressure), HS Mode ON

Table 2. **HS Conditions.**

Headspace System	**TurboMatrix 110 HS Trap in standard HS mode (trap port capped).**
Vial Equilibration	90 °C for 20 minutes
Needle	130 °C
Transfer Line	140 °C, long, 0.150 mm i.d. fused silica (chosen to facilitate rapid conversion to HS trap operation for other applications)
Carrier Gas	Helium at 35 psig
Injection Time	0.15 min

Table 3. **MS Conditions.**

Mass Spectrometer	**Clarus SQ 8 MS, Large Turbo Pump**
Scan Range	35 to 350 Daltons
Electron Energy	70eV
Scan/Dwell Time	0.1 s
Interscan/Interchannel Delay	0.02 s
Source Temp	200 °C
Inlet Line Temp	200 °C
Multiplier	1400V

Table 4. **Sample Details.**

Sample	10.00 ±0.01 g of olive oil weighed directly into vial
Vial	Standard 22-mL vial with aluminum crimped cap with PTFE lined silicon septum

Calibration solutions

1 mL of each BTEXS component was added to a 100-mL volumetric flask and diluted to volume with methanol. 1 mL of this stock solution was further diluted to 100 mL with methanol to produce the working solution used to fortify 'clean' olive oil for calibration purposes. The w/v concentration of each analyte in each of these two solutions is given in Table 5.

Table 5. **BTEXS concentrations in calibration solutions.**

Component	**Stock Solution (µg/µL)**	**Working Solution (ng/µL)**
Benzene	8.77	87.7
Toluene	8.70	87.0
Ethylbenzene	8.67	86.7
p-Xylene	8.80	88.0
m-Xylene	8.64	86.4
o-Xylene	8.80	88.0
Styrene	9.06	90.6
Methanol	Balance	Balance

Experimental

Method Optimization

Figure 1 shows a total ion chromatogram (TIC) obtained from an empty vial into which 2 µL of the working mixture of the BTEXS components in methanol was injected and fully evaporated. The conditions given in Tables 1 to 3 were applied.

Excellent peak shape is apparent and a full baseline separation of all components has been achieved. Meta-xylene and para-xylene are easily separated on this highly polar chromatographic column. A solvent delay of 4.6 minutes eliminates the appearance of the methanol solvent peak in this chromatogram.

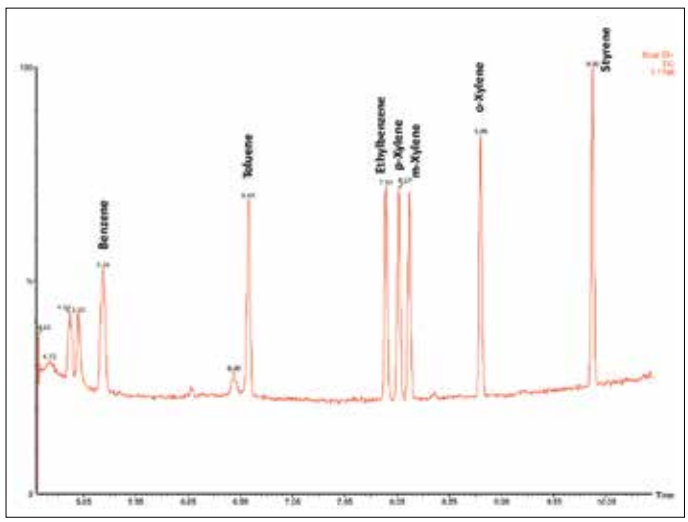

Figure 1. Chromatogram of 2 µL of working calibration solution added to an empty 22-mL HS vial.

Figure 2 shows a chromatogram (with the same scaling as Figure 1) run under the same analytical conditions of 2 µL working calibration mixture mixed into a 10 g sample of 'clean' olive oil. The analyte peaks are either close to the background noise level or are obscured by other components. The effective concentration of each analyte in the oil is approximately 17 ng/g (or ppb w/w). We need to see levels below 5 ng/g with this analysis and so it is clear that this will be a challenge with the method used to produce this chromatogram. The BTEXS compounds obviously have an affinity for the olive oil and so the partition coefficients are not favorable to the headspace phase – only a very small fraction of these will make it into the headspace.

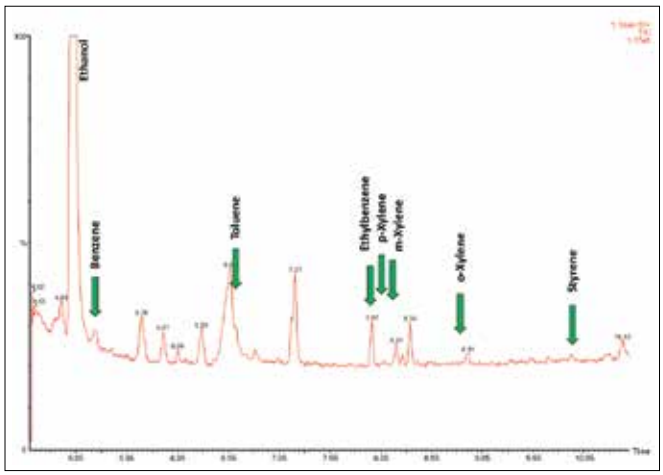

Figure 2. Chromatogram of 17 ng/g BTEXS in 10 g olive oil in a 22-mL HS vial with expected analyte retention times annotated.

By using the MS single-ion recording (SIR) mode of operation, the detector sensitivity and selectivity is significantly enhanced as shown in Figure 3. This chromatography was produced using the same chromatographic conditions as for Figure 2 but with the mixed single ion/full ion (SIFI) regime given in Figure 4 applied.

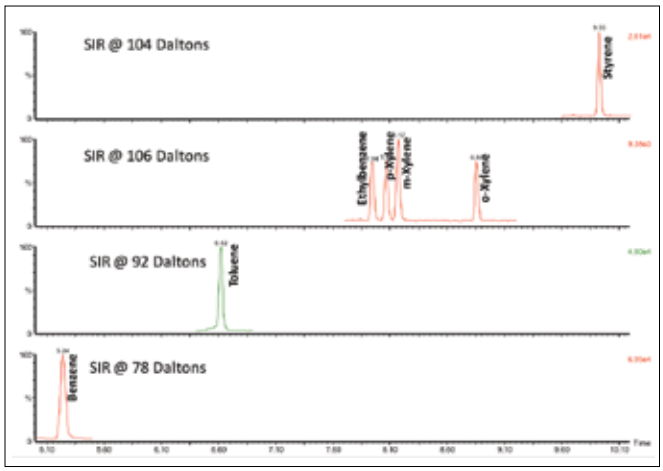

Figure 3. Chromatogram of 17 ng/g BTEXS in 10 g olive oil using SIFI settings given in Figure 4.

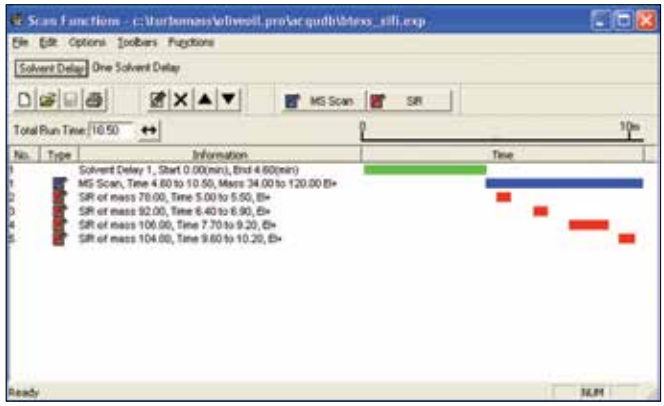

Figure 4. SIFI™ settings used to produce the chromatography shown in Figure 3.

Linearity

A series of calibration mixtures was prepared by adding volumes of the working solution to clean olive oil as listed in Table 6. Note – this is often referred to as "method of standard addition".

Table 6. **Calibration mixture preparation.**

Olive Oil (g)	Working Solution (µL)	Nominal Concentration (ng/g)
10.00	0	0
10.00	0.5	4.4
10.00	1.0	8.8
10.00	2.0	17.6
10.00	3.0	26.3
10.00	4.0	35.1
10.00	5.0	43.9
10.00	10.0	87.8

These mixtures were chromatographed using the conditions given in Tables 1 to 3. The analyte peak areas were obtained from the SIR traces. The clean olive oil was an off-the shelf product found to have low levels of BTEXS. The analyte peak areas found in this oil were subtracted from the calibration mixture responses, which were then used to prepare linear calibration profiles.

Figures 5 and 6 show calibration plots for the first and last eluting analytes, benzene and styrene, and Table 7 shows the least squares fit for each analyte. The linearity is excellent across this low concentration range especially for a complex sample matrix like olive oil.

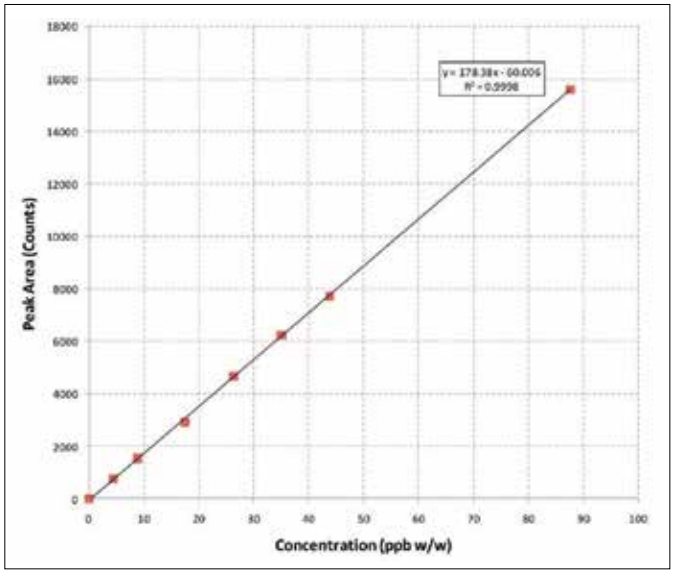

Figure 5. Calibration plot for benzene.

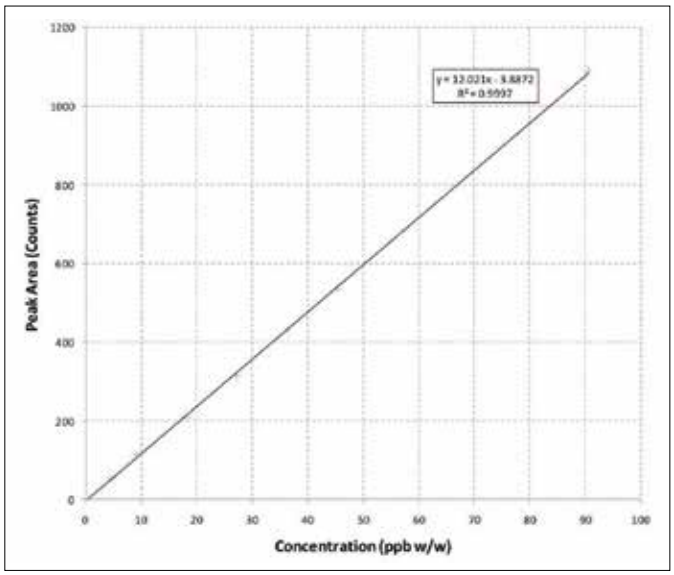

Figure 6. Calibration plot for styrene.

Quantitative Precision

Ten samples of the clean olive oil were fortified with 5 µL of the working solution. Each was analyzed using the conditions given in Tables 1 to 3 and the amount of each analyte was determined using the calibration data from Table 7. The results are given in Table 8. An overall precision of 1.69 to 3.76% relative standard deviation is a very good result from this complex matrix.

Detection Limits

Figure 7 shows chromatography of a low-level sample. The calculated signal to noise ratios were used to predict the analytical detection limits shown in Table 9 based on a 2:1 ratio. These limits are over an order of magnitude below that of the 5ng/g requirement.

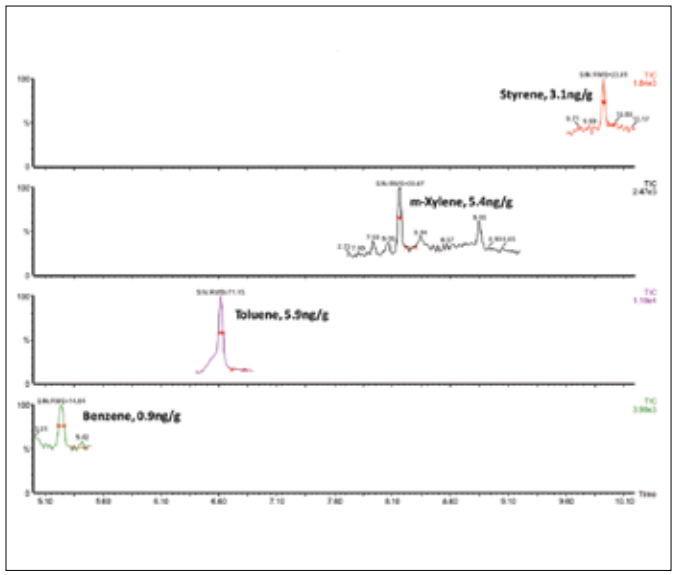

Figure 7. Chromatography of a sample containing low-levels of BTEXS with annotated signal to noise values.

Table 7. **Least squares linear fit to calibration data.**

Statistic	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
Slope	178.38	51.465	10.07	11.568	10.708	8.4239	12.021
Intercept	-60.006	-1.6527	-5.6768	-6.7959	-1.1014	-6.7186	-3.8872
r^2	0.9998	0.9986	0.9995	0.9997	0.9998	0.9995	0.9997

Table 8. **Quantitative precision.**

Run #	Concentration in Spiked Sample (ng/g)						
	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
1	42.84	48.01	43.17	41.05	44.09	43.53	43.83
2	42.60	46.35	44.46	42.95	46.24	45.43	45.16
3	44.27	47.42	45.45	44.85	49.32	46.98	48.32
4	43.30	47.17	44.85	42.51	46.98	45.55	45.66
5	42.87	45.44	43.56	40.09	44.65	44.25	45.16
6	42.40	43.83	43.66	40.27	44.18	42.46	42.75
7	42.90	49.37	44.56	41.91	45.49	44.01	45.25
8	43.30	45.03	44.85	42.08	45.95	44.13	44.66
9	41.91	44.18	43.37	40.35	44.37	43.65	44.33
10	41.77	46.41	42.17	41.30	44.18	42.23	42.92
Mean	42.82	46.32	44.01	41.74	45.54	44.22	44.81
RSD%	1.69	3.76	2.25	3.51	3.66	3.26	3.53

Table 9. **Predicted limits of detection.**

Compound	Predicted Limit of Detection (ng/g)
Benzene	0.12
Toluene	0.16
Ethylbenzene	0.26*
p-Xylene	0.26*
m-Xylene	0.26
o-Xylene	0.26*
Styrene	0.26

** Peaks too small to quantify and so are based on value for m-Xylene.*

Sample Analysis

Seven different branded bottles of olive oil were purchased from a local supermarket and analyzed using this method. The results are given in Table 10. The determined concentrations are well within the range of this method.

Table 10. **Results from analysis of supermarket samples.**

Sample Source(s)	Concentration in Sample (ng/g)						
	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
California	0.89	5.86	1.66	1.45	5.24	3.77	3.07
Italy, Greece, Spain, Tunisia	2.86	27.55	6.12	5.86	16.73	8.75	41.34
Italy, Spain, Greece, Tunisia	3.07	24.22	13.47	7.85	23.64	13.97	39.59
Italy, Spain, Tunisia, Turkey, Argentina	2.99	17.03	3.74	3.44	9.35	6.14	40.09
Spain, Argentina	2.43	34.99	7.22	7.42	18.97	10.65	126.11
Italy, Spain, Greece, Tunisia, Morocco, Syria, Turkey	4.09	35.71	19.13	17.10	59.31	28.10	61.05
Italy, Greece, Spain, Tunisia	1.25	2.79	ND	1.80	3.74	3.17	7.39

Conclusions

This method uses the new Clarus SQ 8 GC/MS to great effect. Sample preparation is extremely easy – 10 g of olive oil is weighed into a standard headspace vial and then sealed with a crimped cap. The analysis is fully automated and takes just 10.5 minutes for the chromatography and an additional 3.5 minutes for cool-down and equilibration between analyses.

Sub-ppb levels are possible using standard headspace sampling of light aromatics in a complex natural oil matrix without the need for vapor pre-concentration (for example with an HS Trap). Excellent quantitative performance has been demonstrated and the system is easily able to see low concentrations of these compounds in olive oil bought from a local supermarket.

PerkinElmer Accessories and Consumables for this application:

Item Description	Part No.
Elite Wax	N9316485
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Ferrules	09920104
H/S Vials/Caps/Septa	N9303992
Marathon Filament	N6470012
Ergo Crimper	N6621037

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APPLICATION NOTE

Mass Spectrometry

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Rapid Measurement of Olive Oil Adulteration with Soybean Oil with Minimal Sample Preparation Using DSA/TOF

Introduction

Among edible oils, olive oil shows important and outstanding characteristics due to its differentiated sensorial qualities (taste and flavor) and higher nutritional value. It is an important oil that is high in nutritional value due to its high content of antioxidants (including vitamin E)[1]. Several health benefits, such as its ability to lower LDL cholesterol and its anti-inflammatory activity, associated with its consumption were initially observed among Mediterranean people[2,3]. Olive oil is one of the most adulterated food products of the world due to its relatively low production and higher prices as compared to vegetable and seed oils.

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Olive oil and other oils are composed mainly of triacylglycerols. These molecules are derived from the esterification of three fatty acid molecules with a glycerol molecule and these fatty acids determine the fatty acid composition of oils. Olive oil contains more oleic acid and less linoleic and linolenic acids than other vegetable and seed oils. Oleic acid is a monounsaturated fatty acid, whereas linoleic and linolenic acids are polyunsaturated fatty acids. The main fatty acids in olive oil are: oleic acid (65-85%), linoleic acid (4-15%), palmitic acid (7-16%) and linolenic acid (0-1.5%). The main fatty acids in soybean oil are: oleic acid (19-30%), linoleic acid (48-58%), palmitic acid (7-12%) and linolenic acid (5-9%)[4,5]. Therefore, the ratio of linoleic and linolenic acid to oleic acid in olive oil can be used as a way to detect its adulteration with soybean oil and other seed oils such as corn, safflower, sunflower and sesame oil, which have a higher content of linoleic and linolenic acids and lower amount of oleic acid in comparison to olive oil[6]. Using this strategy with the AxION® Direct Sample Analysis™ Time-of-Flight mass spectrometry system (DSA/TOF), we detected adulteration of olive oil with soybean oil.

The addition of vegetable and seed oils of low commercial and nutritional value to olive oil results in frequent problems for regulatory agencies, oil suppliers and consumers. A lot of scientific effort has been spent to develop rapid, reliable, cost effective analytical approach for measurement of adulteration of olive oils with other oils. In the past, methods employing gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC) hyphenated to MS (HPLC/MS) have been implemented for this purpose[7,8,9,10]. These methods are time consuming, expensive and require extensive sample preparation, method development and derivatization. In this work, we demonstrated that the AxION Direct Sample Analysis (DSA™) system integrated with the AxION 2 Time-of-Flight (TOF) mass spectrometer can be used for rapid screening of adulteration of olive oil with soybean oil. The advantages of this method, compared to conventional techniques, are that no chromatography is required, the combination of direct sampling from the olive oil is done with minimal or no sample preparation and mass spectra results are generated in seconds.

Experimental

Olive oil and soybean oil were purchased from a local supermarket. Both oils were diluted to 1% in iso-propanol with 10 mM ammonium acetate. After dilution, the oils were mixed in different proportions to simulate the adulteration of olive oil with soybean oil at different percentages of 5, 10, 25 and 50. All oils and their mixtures were measured with an AxION 2 DSA/TOF system with minimal sample preparation. Five µl of each sample was pipetted directly onto the stainless steel mesh of the AxION DSA system for ionization and analysis. The DSA/TOF experimental parameters were as follows: corona current of 5 µA and heater temperature of 350 °C. The AxION 2 TOF MS was run in negative ionization mode with flight voltage of 8000 V and capillary exit voltage of -120 V for the analysis. Mass spectra were acquired in a range of m/z 100-700 at an acquisition rate of 5 spectra/s. All samples were analyzed within 30 sec. To obtain excellent mass accuracy, the AxION 2 TOF instrument was calibrated before each analysis by infusing a calibrant solution into the DSA source at 10 µl/min.

Results

Figure 1 and Figure 2 show the mass spectra for a 1% solution of olive oil and soybean oil in iso-propanol with 10 mm ammonium acetate in negative ion mode using DSA/TOF, respectively. The mass spectra shows that the fatty acids, oleic, linoleic and linolenic, are present in both oils, but their relative amount is different in the two oils. The data shows that the response ratio for linoleic acid to oleic acid (L/O) was 0.18 and 1.86 in olive oil and soybean oil, respectively. Also, the response ratio for linolenic acid to oleic acid (Ln/O) was 0.017 and 0.29 in olive oil and soybean oil, respectively. Therefore, the higher response ratio for linoleic and linolenic acid to oleic acid can be used to detect adulteration of olive oil with soybean vegetable oil using DSA/TOF. This is supported further by data in Figure 3 which shows that response ratio of linoleic and linolenic acid to oleic acid was higher roughly by a factor of 2 for olive oil adulterated with 10 % soybean vegetable oil in comparison to olive oil. Figures 4 and 5 show that the response ratio for linoleic acid and linolenic acid to oleic acid increased, with

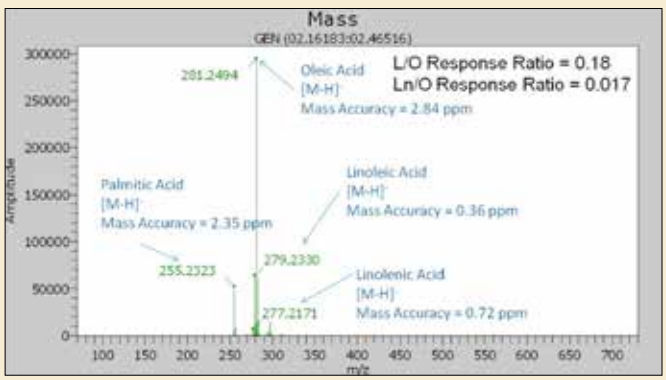

Figure 1. Mass spectra of olive oil diluted by a factor of 100 in negative mode using DSA/TOF.

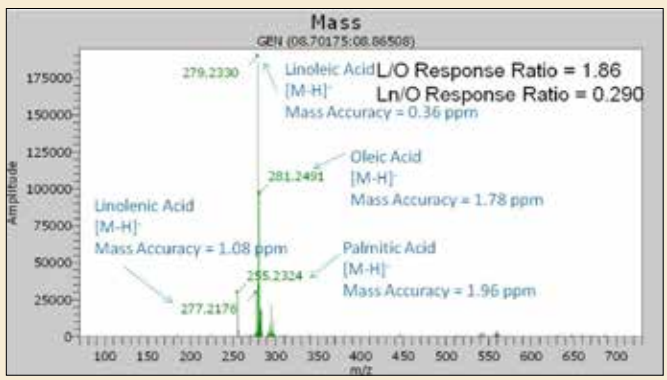

Figure 2. Mass spectra of soybean oil diluted by a factor of 100 in negative mode using DSA/TOF.

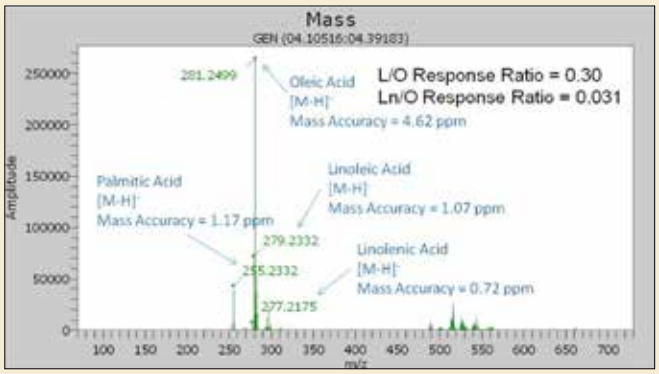

Figure 3. Mass spectra of olive oil adulterated with 10 % soybean oil diluted by a factor of 100 in negative mode using DSA/TOF.

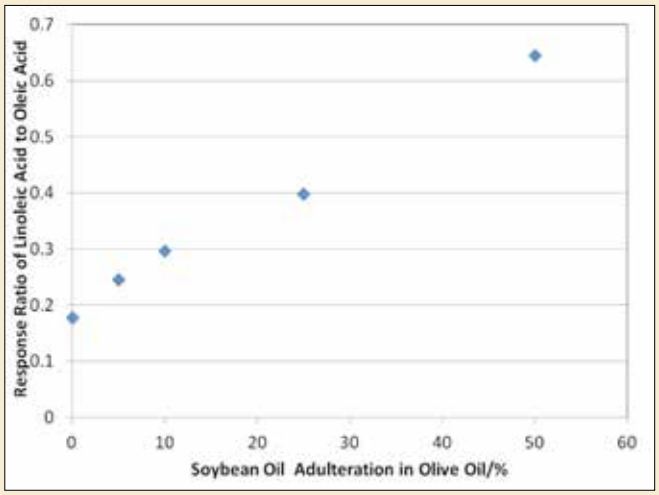

Figure 4. Effect of olive oil adulteration with different levels of soybean oil on response ratio of linoleic acid to oleic acid.

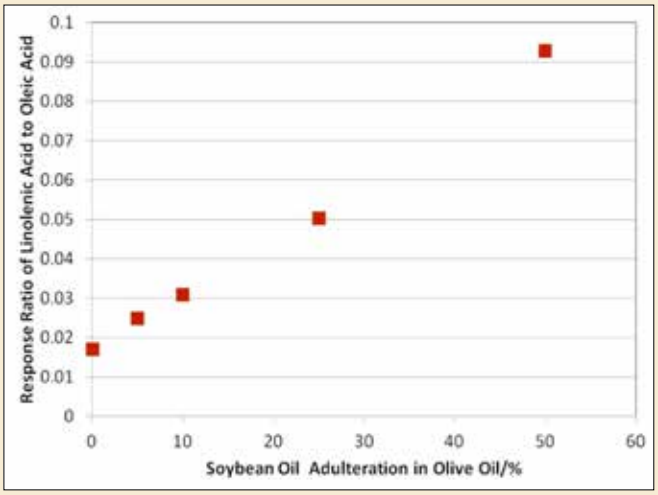

Figure 5. Effect of olive oil adulteration with different levels of soybean oil on response ratio of linolenic acid to oleic acid.

an increase in adulteration of olive oil with soybean oil from 5 to 50 %. This further confirmed that adulteration of olive oil with soybean oil can be detected by measuring the response ratio for linoelic and linolenic acid to oleic acid with DSA/TOF. All mass measurements showed good mass accuracy with an error of less than 5 ppm.

Conclusion

This work shows the first work for rapid screening of adulteration of olive oil with soybean oil using DSA/TOF. The data showed that the higher response ratio for linoleic and linolenic acid to oleic acid in olive oil can be used to detect its adulteration with soybean oil. The mass accuracy of all measurements was less than 5 ppm with external calibration. All samples were screened with minimal sample preparation, in 30 sec per sample. In comparison to other established techniques such as LC/MS and GC/MS, DSA/TOF will improve laboratory productivity and decrease operating costs and analysis time.

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APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Differentiation of Olive, Vegetable and Seed Oils by LC/MS Analysis of Triacylglycerides

Introduction

Extra virgin olive oil is known for its nutritional value, high antioxidant content and low saturated fat content when compared to other oils.[1] This expensive oil has been adulterated throughout history with less expensive oils such as soybean or hazelnut oil. Detecting adulteration is important for maintaining both the safety and consumer confidence of this product.

This study reports on the measurement of the triacylglyceride composition of a large number of olive oils and other seed and vegetable oils using a simple LC/MS method, together with statistical analysis of the results to find indicators for different oil types.

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Method

Oil Samples

A number of different seed and vegetable oils, together with samples of virgin and refined olive oils, olive pomace oil, light olive oil and extra virgin olives oils (EVOOs) from California and Spain were analyzed. Samples were purchased from supermarkets or donated by suppliers.

LC-TOF-MS Analysis

The triglycerides (TAG) levels of the oils were detected using a positive mode LC/MS analysis method. Because TAGs are the main constituents of olive oils, only a simple dilution of the oils in acetonitrile was required prior to analysis.

Samples were analyzed with a reversed-phase separation using a 2 mm x 10 cm Brownlee SPP C18 column and an acetonitrile/ethyl acetate gradient at a flow rate of 0.4 mL/min from a Flexar™ FX-10 UHPLC pump. A post-column addition of a 10 mM aqueous ammonium formate solution at 0.2 mL/min through a T-fitting was used to promote the formation of $[M+NH_4]^+$ ammonium adduct ions. TAGs were detected with positive mode electrospray ionization on an AxION® 2 TOF MS fitted with an Ultraspray™ 2 ion source. Each of the oils were analyzed in triplicate.

PCA Analysis

All of the LC/MS datasets were processed in a batch with a proprietary algorithm to extract the intensities for the significant features in each dataset. Each feature is produced by a chemical component with a distinct m/z value and a profile in the time domain that is consistent with the typical LC peak widths for that HPLC column.

The processing results are exported into a table, with rows for each dataset and intensities aligned into columns for each feature; the column headings are a text summary of rounded m/z and retention time values for that feature.

TIBCO Spotfire® was used for statistical analysis of the data table using an S-Plus Principal Component Analysis function and for graphical display of result in scatter plots and bar charts. The intensities in the table are the variables used for the statistical analysis. The column headings are used for labels of features in plots.

Categories such as oil type, country of origin and olive type for each sample were linked to the table. This enabled different grouping and sorting options and color coding of graphs.

PCA transforms or projects the variables for each sample into a lower dimensional space while retaining the maximal amount of information about the variables. Resulting principal components for each sample are a combination of the original variables after the transformation. The largest difference in the combined variables between the samples is described by Principle Component 1 (PC1), the next largest by PC2 and so on.

Scores Plots of principal components are used to investigate the relationship between samples. Samples that group together have similar levels of variables. A Loadings Plot is a means of interpreting the patterns seen in a Scores Plot, with variables furthest from the origin having the most significant contribution to sample grouping.

Results and Statistical Analysis of a Small Set of Californian EVOO, Seed and Vegetable Oils

An initial small scale study of a number of Californian EVOOs and adulterant oils was made to test out the validity of the TAG analysis method.

PCA of the TAG features in the datasets results in a Scores Plot for PC1 v. 2 (Figure 1 left panel) showing a close grouping of all olive oil samples and complete separation of this group from other oil types. The soybean, grape seed and sunflower oils are widely separated from the EVOOs along the PC2 axis, with canola oil less separated on the same axis.

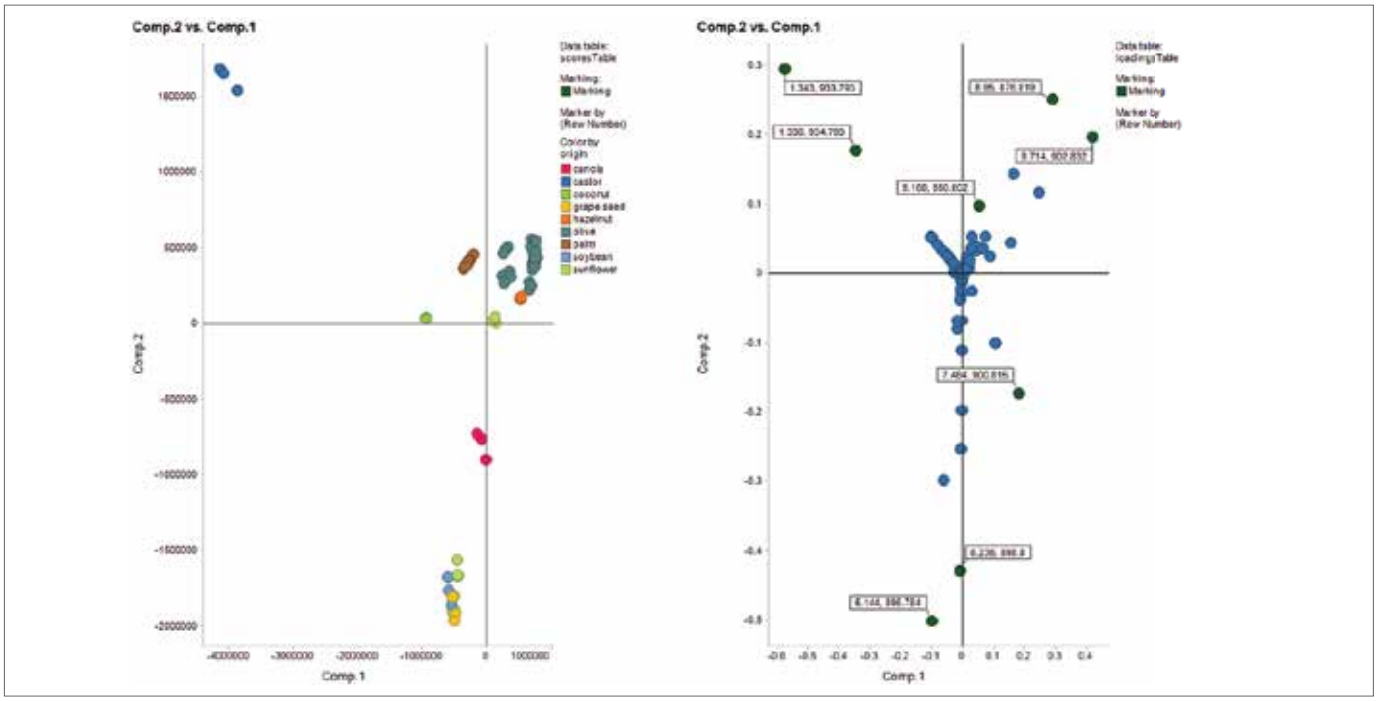

Figure 1. PCA Scores and Loadings Plots of the TAG levels with samples colored by oil type. Only California EVOO oils, shown in blue-green, are included in this initial analysis. Most significant loadings variables are m/z 896, 898, 933, and 876.

The loadings plot (Figure 1 right panel) shows the features on the PC2 axis which most influences this grouping.

Each feature is labeled with a rounded m/z value and retention time created by the extraction algorithm. The identification of all the TAG ions was confirmed from the accurate masses of the molecular ions in peaks in the original datasets. The detected TAGs are listed in Table 1.

In this Loadings Plot, soybean, grape seed and sunflower oils have higher levels of the TAGs at LLL and LLO than other oils, with canola oil also showing elevated levels.

Table 1. The detected TAGs which are most significant to the sample grouping by cultivar.

m/z	time/min	TAG
902.8171	8.71	OOO
900.8015	7.42	OOL
898.7858	6.29	LLO
876.8015	8.93	OOP
874.7858	7.60	OOPo/POL
850.7858	9.19	OPP
848.7702	7.74	OPPo
933.7753	1.34	$[M+H]^+$ for RRR

m/z for $[M+NH_4]+$ ions of the TAGs
Fatty acid residues in the TAGs indicated as S= stearic, O = oleic, P = palmitic, L = linoleic, Ln= linolenic, Po= palmitoleic, R=ricinoleic

Castor oil is widely separated from all other oils in the upper left quadrant of the Scores Plot. The variable in the same direction in the Loadings Plot is a triglyceride of the hydroxylated long chain fatty acid ricinoleic acid. Castor oil is the only oil known to yield this TAG[2].

Palm and coconut oils group closely to the olive oils in the center of the PC 1 v. 2 Scores Plot. In a different projection of the PCA scores in a Score Plot for PC 3 v. 2 (Figure 2) these two oils are separated from EVOOs due to increased levels of OPP and OPPo.

Tree nut oils are known to have a higher proportion of C16 acid (palmitic and palmitoleic acids) containing TAGs, when compared with olive oils.

Hazelnut oil was not well separated from the olive oils in these initial PCA Scores plots, since both oils have similar levels of all the measured TAGs.

The statistically significant separation of most potential adulterant oils from the olive oils by the pattern of TAGs shows that this information could be used to confirm the oil type and detect adulteration of olive oils with these oils.

Results for a Large Scale Study of Oils Including Spanish EVOOs

A larger study was performed with 35 different monovarietal and blended Spanish EVOOs, together with lower grade olive oils. These results were combined with those from the initial analyses of Californian EVOOs and seed and vegetable oils. This larger group of oils was used to confirm and expand on the earlier findings.

Analyzes of samples were conducted in batches over several days, so the initial PCA Scores Plot of PC 1.v 2 shows differences between sample groups caused by small variations in analysis conditions. The PCA was recalculated using scaled variables; the intensities for each variable were scaled by a factor derived from the sum of the intensities for all variables for that sample.

In the scaled PCA Scores Plot of PC 2 v. 1, oils in the expanded sample set are grouped by oil type (Figure 3). EVOOs, refined and regular olive oils are grouped closely together and most adulterant oils are separated from them, in agreement with the earlier study.

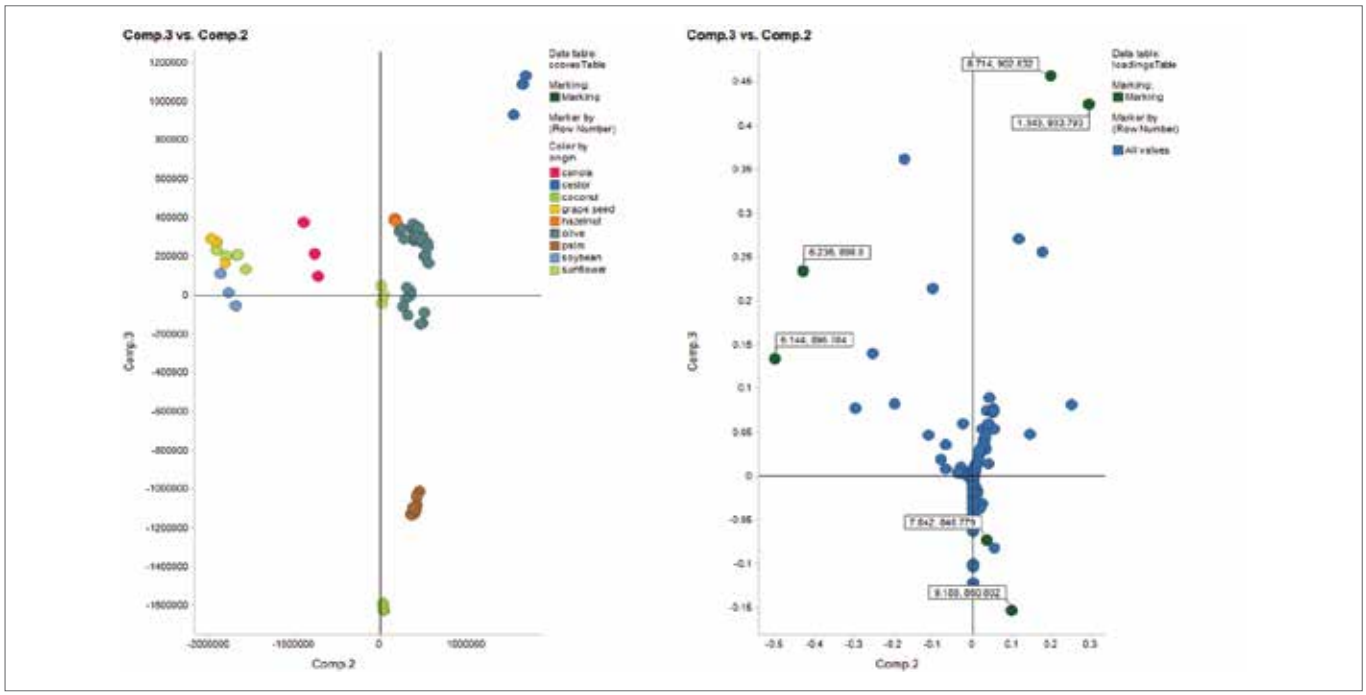

Figure 2. PCA Scores and Loadings Plot of PC 3 v. 2 of Californian olive and other oils. In this projection the palm and coconut oils are separated from the olive oils by higher levels of the TAGs OPP and POPo.

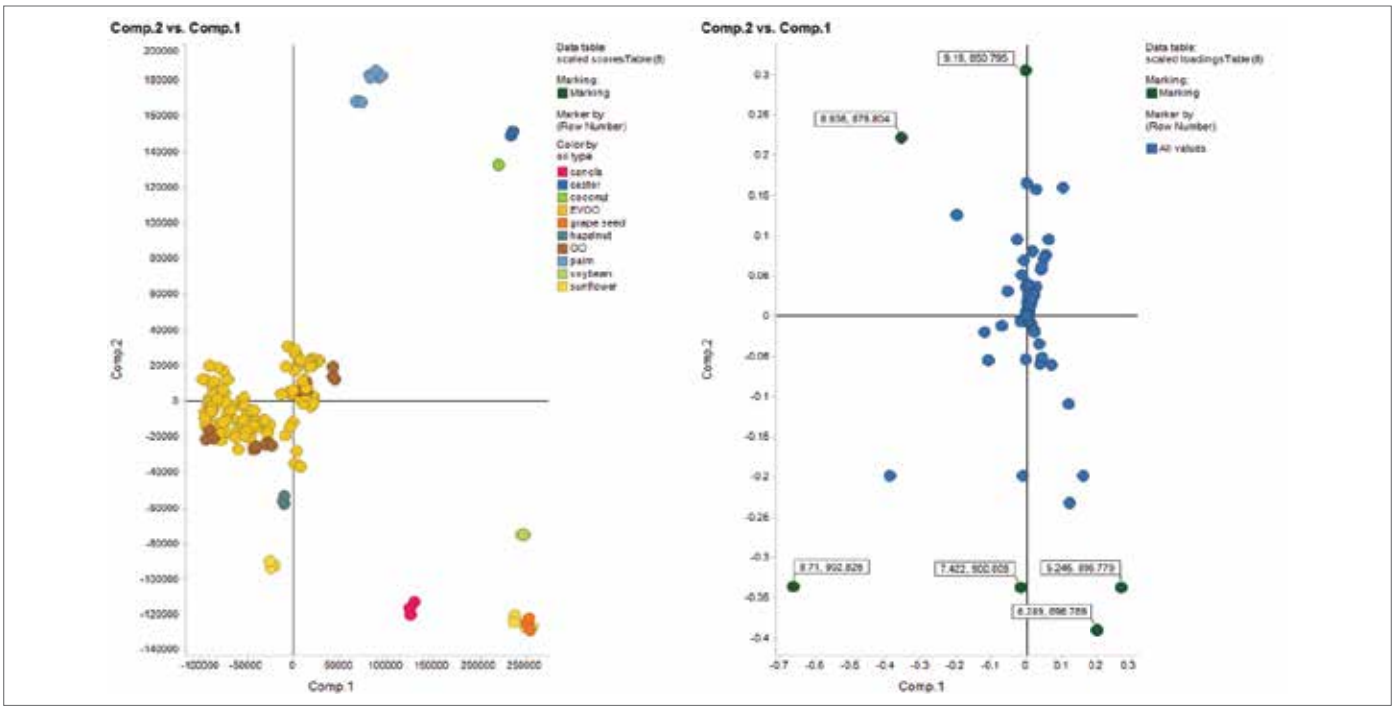

Figure 3. Scores and Loadings Plots of PCA of using scaled variables shows that Spanish and Californian EVOOs in yellow and other olive oils in brown are grouped and separated from other oil types.

Hazelnut oil is separated from all of the olive oil samples in this PCA Scores projection, but has a similar TAG profile to the high oleic acid sunflower oil. The Loadings Plot shows that both of these oils have lower levels of PPO, PLO and POO than the olive oils, but higher levels of OOO.

In a different PCA Scores projection, the Scores Plot of PC 6 v. 2 (Figure 4) shows separation of hazelnut and high oleic acid sunflower oils from olive oils. The differences are due to the levels of the TAGs OPP, OOPo, OOP and OOO in these oils as shown in the Loadings Plot.

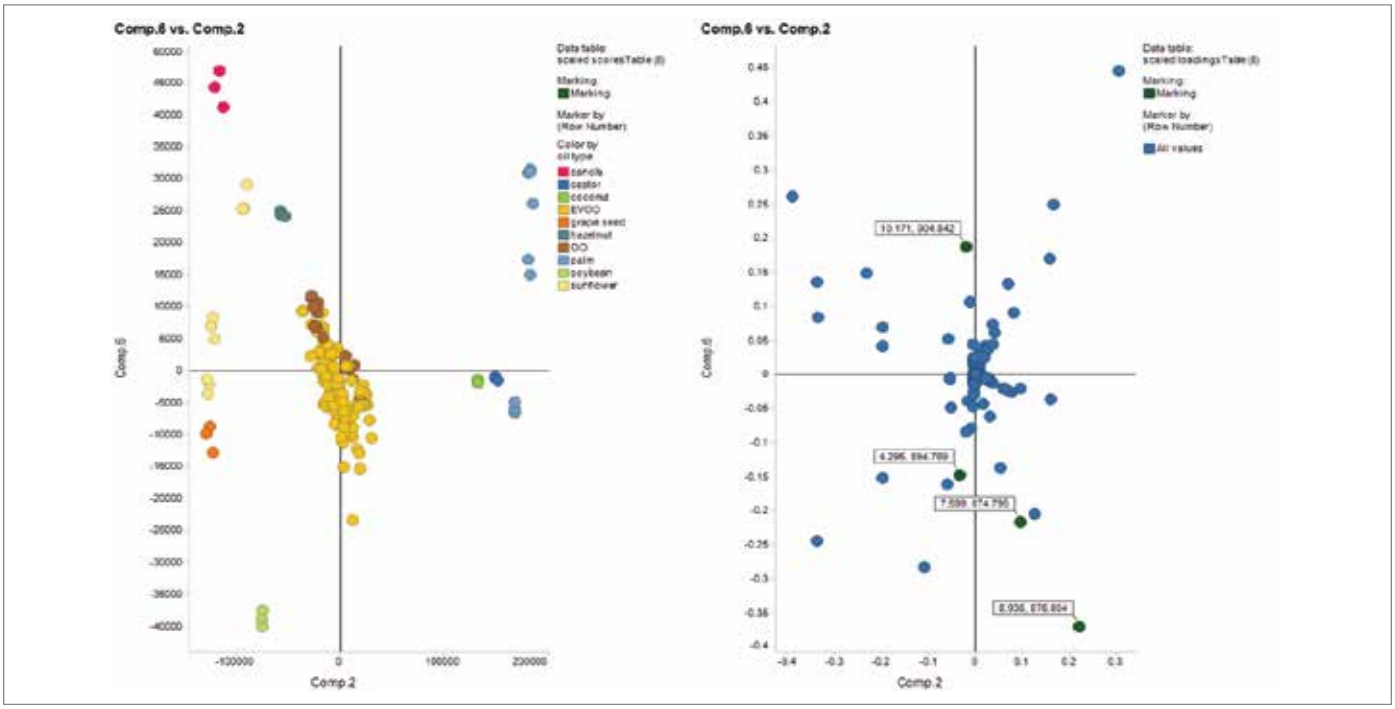

Figure 4. Scores and Loadings Plots of PC 6 v. 2 showing separation of oils. Hazelnut oil is separated from all of the olive oils and also from high oleic acid sunflower oil.

The PCA results suggest that hazelnut oil has a unique profile for these TAGs. This information could be used to detect adulteration of olive oil with hazelnut or sunflower oil, two of the most commonly used adulterants. For example, a bar chart of the ratio of PPO to the most abundant TAG OOO (Figure 5) shows a distinctly different ratio for the adulterant oils. The ratio can be used as a marker for the presence of these adulterant oils in olive oils.

Conclusion

A fast LC/MS method with a minimum of sample preparation was used to detect the TAGs profiles of a large number of seed, vegetable and olive oils.

Statistical analysis of the TAG levels found patterns of TAGs which are characteristic for each oil type. This information could be used to find characteristic markers for different types of oils and to investigate adulteration.

A more detailed analysis of the oils from different Spanish olive cultivars will be covered in a separate applications note.

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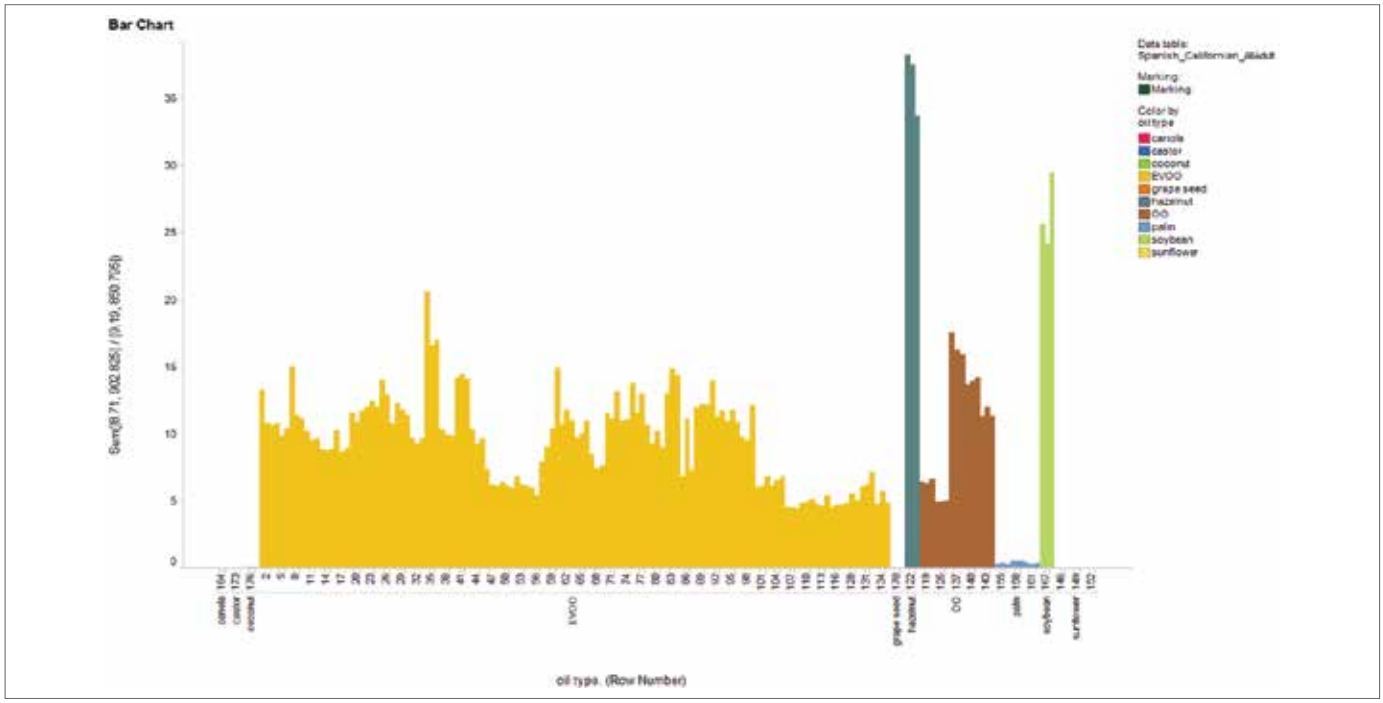

Figure 5. A bar chart of the ratios of OOO to PPO in each oil shows that the hazelnut oil has the highest ratio, with high oleic acid sunflower oil also having a higher ratio than EVOO or refined olive oils.

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APPLICATION NOTE

Mass Spectrometry

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Rapid Measurement of Extra Virgin Olive Oil Adulteration with Olive Pomace Oil with No Sample Preparation Using DSA/TOF

Introduction

Food adulteration or food crime occurs when an ingredient is replaced partially or fully with something different – without the knowledge of the consumer. Most of the time, food adulteration occurs to improve profits by diluting higher value products with lower value materials. The price of extra virgin olive oil (EVOO) is about 3-5 times higher than food grade olive pomace oil and therefore can be found to be blended with lower quality olive pomace oil. EVOO is extracted by mechanical pressing of high quality olive fruit, while olive pomace oil is the residue oil that is extracted by chemical solvents from previously pressed olive mash. Further, olive pomace oil is highly refined to remove chemical impurities. Both EVOO and olive pomace oil have similar triglyceride composition and therefore it is difficult to distinguish them on this basis. In the past, time consuming GC methods with sample preparation have been used to show that the average amount of ethyl ester of oleic acid (EEOA) in extra virgin olive oil and refined lampante olive oil (similar to refined olive pomace oil) is 27.9 mg/kg and 770 mg/kg, respectively[1]. It has also been reported that the ethyl esters of fatty acids in extra virgin olive oil were less than 32 mg/kg, whereas the ethyl esters of fatty acids in olive pomace oil were far higher; in the range of 500-40000 mg/kg[2]. The higher amounts of EEOA in olive pomace oil can be attributed either to heat treatment in processing or to lower quality of olive fruits. Both EVOO and olive pomace oils have similar amounts of free oleic acid (OA)[3]. Therefore, the measurement of ratio of EEOA to oleic acid in EVOO can be used as a way to detect its adulteration with olive pomace oils and other lower quality olive oils, which have a higher content of EEOA in them. In this work using a Direct Sample Analysis™ Time-of-Flight mass spectrometry system (DSA/TOF), we developed a method with no sample preparation to determine adulteration of EVOO with olive pomace oil by measuring the ratio of EEOA to OA.

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Experimental

Five samples of extra virgin olive oils, two samples of olive oil blends and two samples of olive pomace oil were purchased from a local supermarket. All oils were tested without any sample preparation with DSA/TOF. One microliter of each sample was pipetted directly onto a glass tube compatible with the AxION® DSA™ system, for ionization and analysis. All samples were analyzed within 30 seconds of sample introduction.

To obtain high mass accuracy, the AxION 2 TOF mass spectrometer was calibrated before each analysis by infusing a mass calibrant solution into the DSA source at 10 µl/min. The DSA/TOF experimental parameters were as follows:

Mass spectrometer:	PerkinElmer AxION 2 TOF MS
Ionization source:	PerkinElmer Axion Direct Sample Analysis (DSA)
Ionization mode:	Positive
Flight Voltage:	-8000 V
Mass Scan Range:	10-1100 Da
Acquisition Rate:	2 Spectra/s
Data Acquisition Time:	5 s
Capillary exit voltage:	150 V
DSA source temperature:	350 °C
Drying gas flow rate:	4 L/min

Results

Figures 1 and 2 show the mass spectra for the EVOO sample 3 and olive pomace oil sample 2 in positive ion mode using DSA/TOF, respectively. The mass spectra show the response ratio for EEOA to OA were 0.018 and 0.63 in EVOO sample 3 and olive pomace oil sample 2, respectively. Therefore, the higher response ratio for EEOA to OA in EVOO can be used as a way to determine adulteration of EVOO with olive pomace oil using DSA/TOF. Table 1 shows EEOA to OA response ratio for 5 EVOO samples, 2 olive oil blend samples (a mixture of EVOO and olive pomace oil) and 2 olive pomace oil samples. The average response ratio for EEOA to OA in EVOO samples was 0.023 with standard deviation of 0.005. Therefore, if an EVOO sample showed the response ratio for EEOA to OA at a value higher than 0.032 (calculated using value of average + 2 times standard deviation for EEOA to OA response ratio in extra virgin olive oil) with DSA/TOF, it would indicate that it might be either adulterated with olive pomace oil or any oil containing a higher level of EEOA than EVOO. Table 1 also shows that the response ratio for EEOA to OA for 2 olive oil blend samples and 2 olive pomace oils was higher than 0.032, suggesting that these samples were either blends of EVOO and olive pomace oil or olive pomace oil samples. Figure 3 demonstrates that the response ratio for EEOA to OA increased with addition of olive pomace oil sample 2 from 10 to 75 % in extra virgin olive oil 3. This demonstrated further that the higher response ratio of EEOA to OA in EVOO can be indicative of its adulteration with olive pomace oil using DSA/TOF.

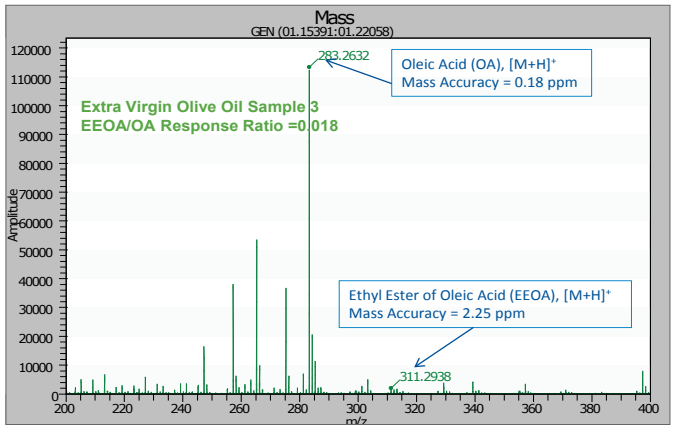

Figure 1. Mass spectra of extra virgin olive oil sample 3 in positive ion mode using DSA/TOF.

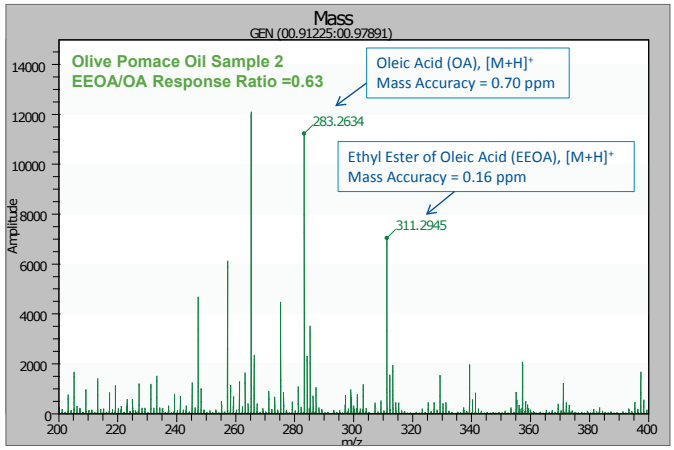

Figure 2. Mass spectra of olive pomace oil sample 2 in positive ion mode using DSA/TOF.

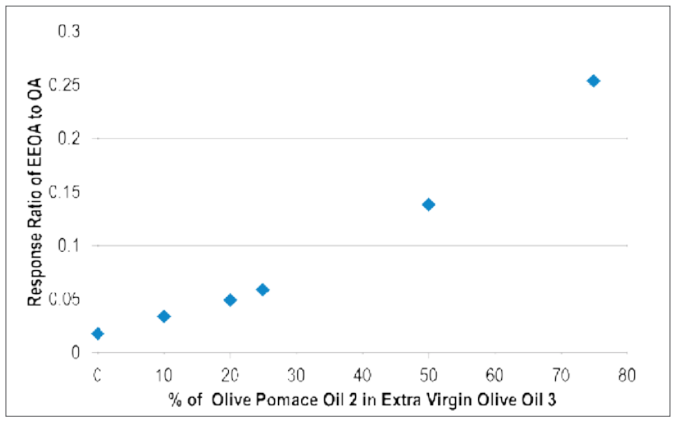

Figure 3. Effect of olive pomace oil adulteration in extra virgin olive oil on EEOA to OA response ratio.

Table 1. Measured values of response ratio of EEOA to OA in different olive oil samples with DSA/TOF.

Sample No.	Sample Description	EOA/OA Response Ratio
1	Extra Virgin Olive Oil 1	0.028
2	Extra Virgin Olive Oil 2	0.023
3	Extra Virgin Olive Oil 3	0.018
4	Extra Virgin Olive Oil 4	0.018
5	Extra Virgin Olive Oil 5	0.028
6	Olive Oil Blend 1	0.083
7	Olive Oil Blend 2	0.037
8	Olive Pomace Oil 1	5.45
9	Olive Pomace Oil 2	0.63

Conclusion

In this application, we developed a rapid method for screening EVOO adulteration with olive pomace oil using DSA/TOF. The data showed that the higher response ratio for EEOA to OA in EVOO can be used to detect its adulteration with olive pomace oil. All samples were tested with no sample preparation and with an analysis time of less than 30 seconds per sample. In comparison to other established techniques such as LC/MS and GC/MS[1-2,4-7], DSA/TOF would decrease operating costs and analysis time.

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APPLICATION NOTE

Differential Scanning Calorimetry

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Detecting the Adulteration of Extra Virgin Olive Oil by Controlled Cooling DSC

Introduction

Food adulteration normally makes the news with cases like melamine in milk[1]. However, high-value products are often subjected to adulteration by lower-value materials and this can be difficult to detect. As a high-priced produce, a pint of extra virgin olive oil (EVOO) is close in cost to that of a half gallon of food-grade olive pomace oil. University of California at Davis has reported that the majority of the extra virgin olive oils sold in California fail the tests for the same (EVOO), using a variety of techniques (ultraviolet and visible spectroscopy [UV/Vis], gas chromatography [GC], liquid chromatography [LC]), and wet methods[2]. However, considering the way EVOO is made, one would expect a relationship to its thermal properties.

Differential scanning calorimetry (DSC) is commonly used to analyze foods in both quality control and research labs[3, 4]. DSC is often used to compare materials on heating, but cooling studies often give more information as materials can respond more thermodynamically under controlled cooling[5].

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Experimental

Materials

Initial samples of four commercial olive oils were obtained locally and then samples of high-grade EVOO were obtained directly from small producers. In addition, samples of freshly pressed mono and multi-varietal EVOO, along with refined and salvage oil with known processing histories, were also obtained. All samples were stored in a cool, dark room, when not used, under N_2 purge.

Instrumental

4-8 mg samples of the various oils were pipetted into pre-weighted and matched aluminum DSC sample pans (PerkinElmer Part No. 02190041). These were then run on a PerkinElmer DSC 8500 under N_2 purge at 20 cc/min and cooled from room temperature to -60 °C at a rate of 5 °C per minute. A two-stage refrigerated cooler was used. Once at -60 °C, they were held there for three minutes to ensure complete cooling. Then, the samples were heated back to room temperature at 10 °C per minute. All samples were run in triplicate and the results averaged.

Figure 1. The DSC 8500 is a dual furnace power compensated design differential scanning calorimeter capable of very precise control on heating and cooling.

Results

The commercial samples of olive oil show distinct thermal differences. Below, extra virgin (solid), refined (dashed), and pomace (dotted) olive oils are shown during the cooling run.

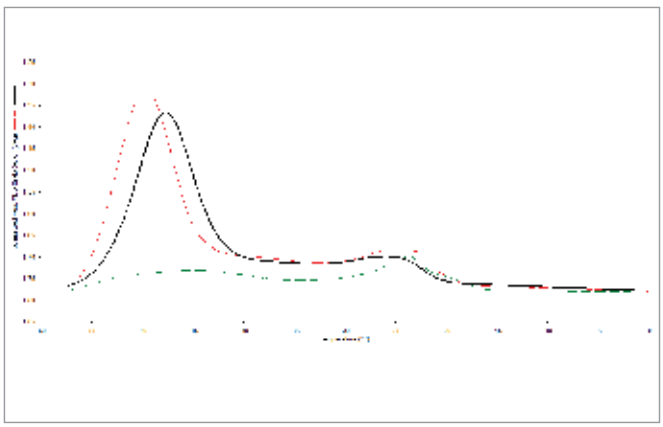

Figure 2. Grocery store grades of extra virgin, refined and pomace olive oils were run by controlled cooling in the DSC. Notice the distinctive fingerprints, particularly of the pomace oil, which lacks the low temperature peak.

As shown in Figure 3, we ran a series of EVOO samples that we were reasonably sure were truly extra virgin, as well as two received directly from a Texas-based producer who could assure this. While preliminary, the data shows some interesting features. First of all, the higher-temperature peak appears in the same temperature range as the pomace oil peak but is very small, even compared to the grocery store EVOO. This data suggests that the grocery store EVOO may be diluted with another oil.

Secondly, it appears that there are shifts in the peak shapes and temperatures with the varietal and origin of the oil. For example, note the difference in shape and peak position of the low temperature peak between the Spanish Arbeguina and the Spanish Arbosana. Origin appears to complicate, as seen in the Texas versus Spanish Arbeguina scans. Futher work would be needed to see if this holds, but based on previous work with nut oils[6], it seems likely.

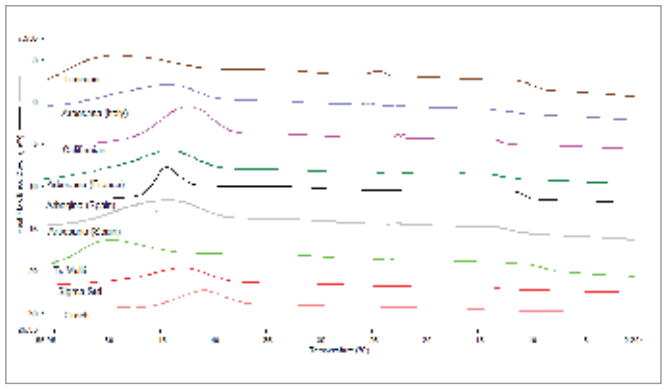

Figure 3. High-quality EVOO from small batch suppliers. The Texas EVOOs were of known origin. Note the lack of strong "pomace peak".

Characterizing these differences is often done by taking partial areas under the curve, as shown in Figure 4. This is shown for EVOO and a large high-temperature peak similar to that of the pomace oil was seen in all the grocery store samples in contrast to the truly EVOO samples in Figure 3.

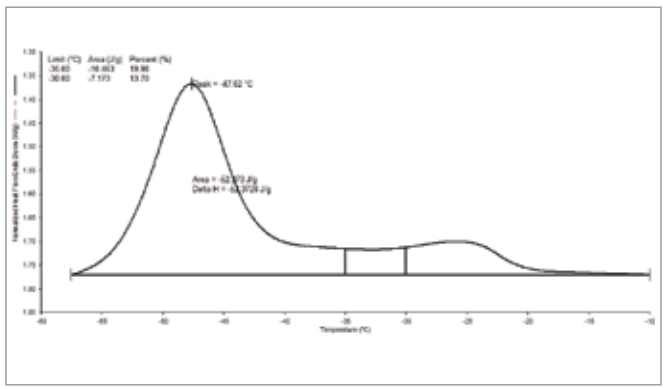

Figure 4. Multiple peak areas in a DSC scan analyzed by the partial areas technique. Only three partial areas are shown above for clarity.

With the "pomace peak" occurring in the -20 °C to -10 °C range and the major "EVOO peak", it appears likely that one could sort materials based on this approach. To test this, we created blends of EVOO and pomace oil in three amounts. The thermograms are overlayed in Figure 5. This data was used to construct a simple model from the partial area data shown above. Linear regression suggests we can estimate the addition of more than 7% olive oil-based adulterant to the olive oil. Based on this approach, we suspect the grocery store EVOO to have 12-15% adulterant or to be pressed at higher temperatures (see Figure 6). More exacting model techniques, such as those used in TIBCO Spotfire® software, are expected to give better results.

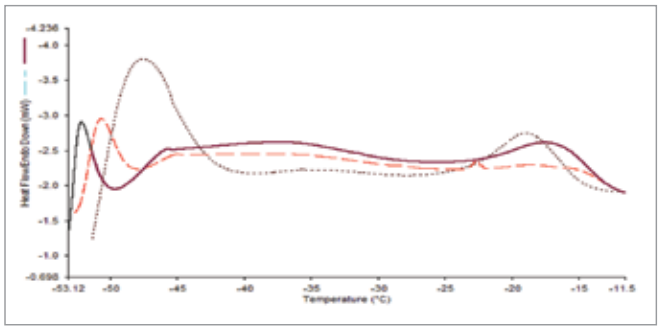

Figure 5. 25% EV (dashed), 50% EV (solid), and 75% EV (dotted) oils during the cooling run.

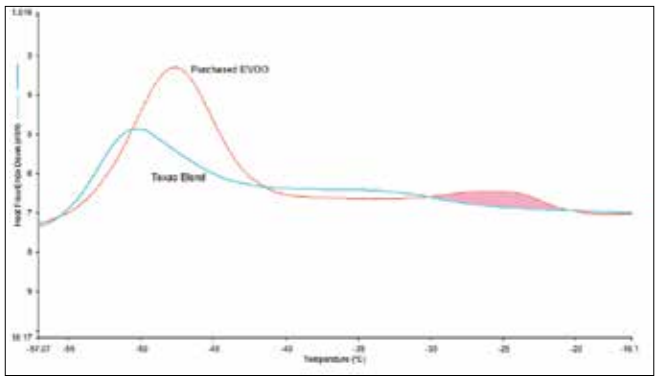

Figure 6. Overlay of the purchased EVOO and the Texas blend of known EVOO. The area of increased "pomace oil" is highlighted.

Conclusion

Controlled cooling in the DSC represents a way to extract information from food products not normally accessible by other methods. Extra virgin olive oils have a distinct cooling profile that is different from lesser grades and apparently this profile is quite responsive to changes in composition. This gives a method for addressing adulterants as well as possibly identifying the varietal used to produce the oil. Futher work is planned on the effect of temperature and UV radiation.

Acknowledgements

We thank Steve Beines of Texas Olive Ranch/Cowgirl Brands for useful discussions and olive oil samples of known history as well as the LAPOM group at University of North Texas for space and support,

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APPLICATION NOTE

FT-IR Spectroscopy

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Advantages of Adulterant Screen for Detection of Olive Oil Adulteration by Attenuated Total Reflectance (ATR) FT-IR

Introduction

Olive oil is an increasingly popular food product worldwide, with consumption in the U.S. alone having increased by about 50% in the last 10 years. Over three million tons annually of olive oil are produced worldwide, with approximately 75% of this being produced in Spain, Italy, and Greece. The U.S. now imports over 300,000 tons of olive oil annually.

Olive oil is considered to be healthy edible oil and is linked to the low incidence of heart disease associated with a Mediterranean diet. It is low in Saturated Fatty Acid (SFA) and Polyunsaturated fats (PUFA) but high in the healthier Monounsaturated fats (MUFA), known to lower cholesterol.

Extra Virgin Olive Oil (EVOO) is a premium product that can command a higher price than "standard" olive oils. This makes it highly susceptible to fraudulent activity. A report by the E.U. Committee on the Environment, Public Health, and Food Safety says olive oil is among the products most prone to food fraud. There were 267 oil adulteration incidents reported to the U.S. Pharmaceutical Food Fraud Database, with the vast majority occurring over the past three years.

Adulteration of EVOO with lower quality olive oils, or other lower cost edible oils, is frequently reported in the media. The most common adulterants include: hazelnut oil, sunflower oil, soybean oil, corn oil, rapeseed oil, and olive pomace oil. Fraudulent activities, such as dilution or even substitution with other lower cost oils containing additional chemicals, that enable the oil to appear to be of higher quality oil and pass routine screening tests are on the rise.

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This application note describes a fast, simple, low-cost solution to screen olive oils for adulteration.

Materials and Methods

Mid-infrared spectroscopy is a well-established technique for the analysis of edible oil samples. The PerkinElmer Spectrum Two™ FT-IR, a high-performance compact FT-IR instrument utilizing the modern ATR sampling technique, offers fast and easy measurements of samples within the food industry. Diamond™ ATR accessories, such as the PerkinElmer Universal ATR (UATR), are extremely robust and allow the instrument to be used in the harshest of laboratories or even in remote environments. The Diamond ATR crystal requires only a very small volume of the sample to be tested and can easily be cleaned between samples, in situ, using laboratory tissue and a small amount of a suitable solvent, such as hexane for edible oils.

In this study the PerkinElmer Spectrum Two, equipped with a UATR sampling accessory, has been used to analyze a series of pure and adulterated olive oils and common adulterant spectra. A typical olive oil spectrum is shown in Figure 2. Spectra were recorded at 4 cm^{-1} resolution with a scan time of one minute per sample.

The prominent features in the spectrum are the bands in the region of 2930 cm^{-1} due to the –CH- stretch of the hydrocarbon chains and in the region of 1740 cm^{-1} due to the carbonyl groups in the triglyceride.

Discriminating Olive Oil from Other Edible Oil Types

The infrared spectra of different edible oils will be similar, only varying by the constituent chains on the triglyceride backbone, since their molecules contain the same chemical groups. However, there are small, observable differences between the different oil types. Figure 3 shows the ATR spectra of three different oil types: olive oil, sunflower oil, and rapeseed (canola) oil.

These spectral differences are significant enough to be able to develop a classification method for these different oils. There are a variety of ways to classify materials based on their infrared spectra. For this type of problem Soft Independent Modeling of Class Analogy (SIMCA), a Principal Components Analysis (PCA) based method, is a good approach to take. Building a SIMCA method requires the measurement of a variety of samples for each type of material you wish to classify. The calibration set of samples should cover all sources of variation normally encountered for that particular material, such as different sources, different batches, or different manufacturing processes. The method will build individual models to completely characterize each of the materials. Each material, in this case the individual oil types, generates its own cluster in this model that should be separated from the other clusters calculated for the other materials being classified. A SIMCA model has been generated for the three types of edible oils in this study. Figure 4 shows the SIMCA model with each oil having its individual cluster, clearly separated from those of the other materials.

Figure 1. The PerkinElmer Spectrum Two and UATR.

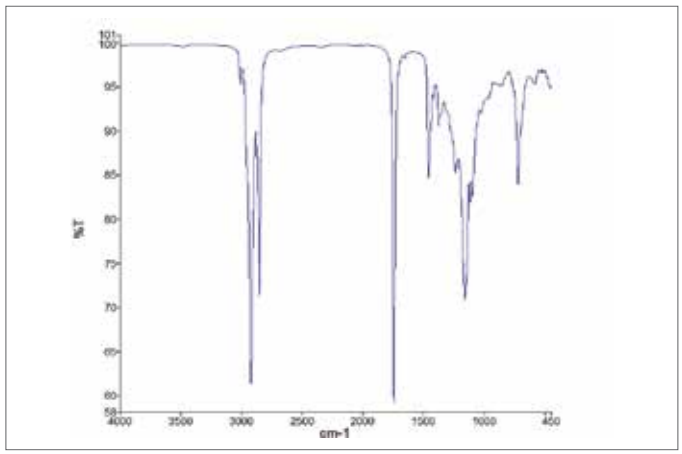

Figure 2. Diamond ATR spectrum of olive oil.

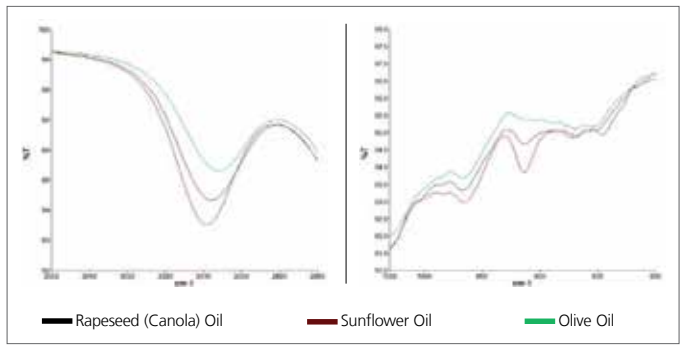

Figure 3. Spectral differences between olive oil, rapeseed oil and sunflower oil.

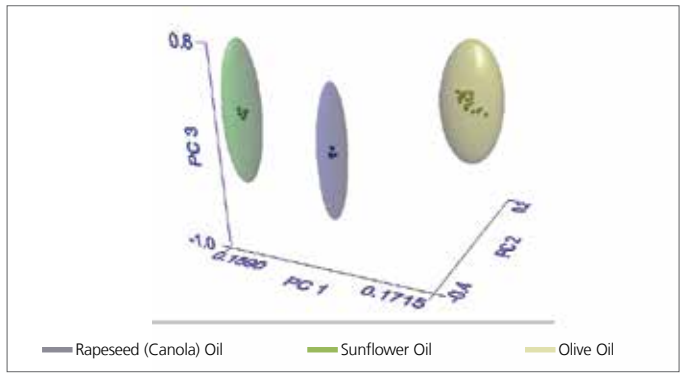

Figure 4. SIMCA model for three edible oil types. Olive oil, rapeseed oil, and sunflower oil.

Classifying a material consists of measuring the IR spectrum and using the SIMCA model to predict to which cluster the spectrum belongs. If the spectrum does not fall into one of the three classes of materials then it is likely to be a different material or contaminated/adulterated oil. Further data investigation would be required to determine the reason that the sample has failed the test.

Quantifying Levels of Known Adulterants in Olive Oil

If the identity of the adulterant is known then it is possible to quantify the amount of adulterant present. This involves the preparation and measurement of the IR spectra of standard mixtures of the olive oil with the adulterant oil. The IR spectra for a series of standards are shown in Figure 5.

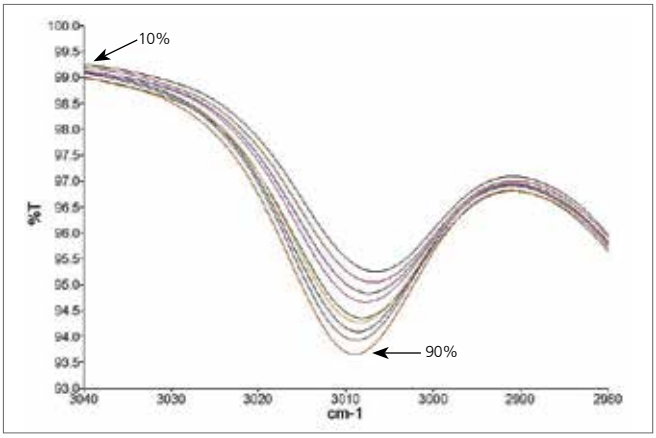

Figure 5. Standards from 10% - 90% Sunflower Oil.

Partial Least Squares (PLS1) Calibrations have been generated for mixtures of olive/sunflower oils and olive/rapeseed oils ranging from 0 to 100% olive oil. The calibrations are shown in Figure 6.

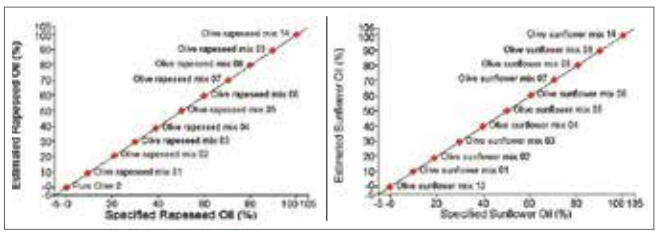

Figure 6. PLS1 Calibrations for Olive/Rapeseed and Olive/Sunflower oils.

An independent validation set of three samples were used to test the calibration model. The validation plot is shown in Figure 7.

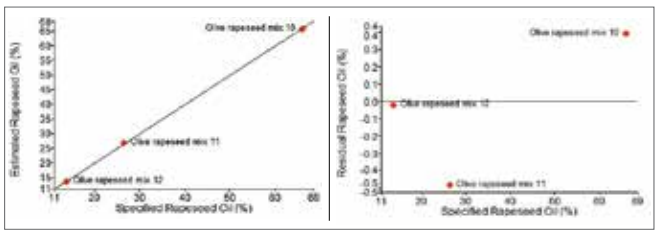

Figure 7. Independent validation samples for olive/rapeseed mixtures.

Adulterant Screen™ Algorithm for Detecting "Known" and "New" Adulterants in Olive Oil

The two statistical approaches taken so far would allow for:
a.) checking that the material is the correct material (SIMCA) and
b.) quantifying the amount of a single, known adulterant (PLS).

An alternative approach is available using an Adulterant Screen Algorithm. The approach is simple:

1. Generate a library of unadulterated material samples spectra exactly as for SIMCA. This library should span as much as possible the natural variation of the material, due to differences between batches, suppliers or processing parameters, etc.
2. Generate spectra of adulterants of concern. These spectra should be of the pure adulterant material, not mixtures. (As new adulterant materials emerge these can easily be added to the adulterant library in the future.)

These two sets of spectra are registered in the software, and the method is ready to use.

In this study, a series of 24 olive oil spectra were measured from commercially purchased oils. These 24 spectra were used to generate a library of the unadulterated material. The objective of this study was to specifically look for adulteration with either sunflower or rapeseed oils. Single spectra of the two adulterants were measured and stored with the method. The Adulterant Screen method was tested using samples adulterated with known concentrations of the other oil types and also with pure olive oil. The results are shown in Table 1.

Table 1. Adulterant Screen results for a series of method validation standards.

Sample Name	Adulterant	Level	Unidentified Components	Adulterant Screen Pass/Fail
Sunflower 18.66% Std	Sunflower Oil	0.19208	Probable	Fail
Sunflower 68.80% Std	Sunflower Oil	0.69011	Probable	Fail
Sunflower 38.10% Std	Sunflower Oil	0.38183	Probable	Fail
Sunflower 100.0% Std	Sunflower Oil	1.00328	Probable	Fail
Rapeseed 66.02% Std	Rapeseed Oil	0.64944	Probable	Fail
Rapeseed 26.41% Std	Rapeseed Oil	0.26367	Probable	Fail
Rapeseed 13.79% Std	Rapeseed Oil	0.14083	Probable	Fail
Rapeseed 100.0% Std	Rapeseed Oil	0.99191	Probable	Fail
Pure Olive Oil	No Adulterants	-	Unlikely	Pass

In all cases, except the pure olive oil, the adulterated samples generated a "Fail" result indicating the presence of an adulterant. Not only does the Adulterant Screen algorithm correctly identify the adulterant, but it also gives an estimated level of that contaminant without the requirement for running quantitative calibration standards. The level of the contaminant is reported as the proportion of the total spectrum contribution arising from that component. The results table demonstrates the ability of this algorithm to classify like SIMCA and additionally provide approximate estimates of concentration of the adulterants without the need to generate extensive quantitative models.

When a sample spectrum is scanned, the algorithm first compares it to a PCA model generated from the reference materials. This model is then augmented with each of the adulterant spectra in turn. If including a given adulterant in the model greatly increases the fit of the sample spectrum, it is likely that the adulterant is actually present in the sample.

Figure 8 shows the residuals observed from the analysis of 13.79% rapeseed validation standard.

Note: the spectral region from 2450-1850 cm^{-1} (the region where the diamond absorptions due to the Diamond ATR are intense) was excluded from the method.

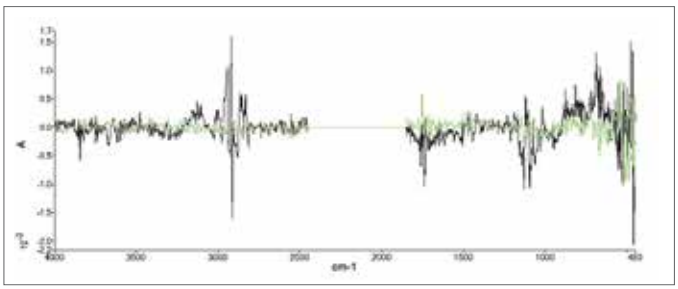

Figure 8. Spectral residuals before (black) and after (green) fitting adulterants.

In this case the residuals are significantly decreased by fitting the spectrum of the pure rapeseed oil indicating the presence of that adulterant in the sample.

Summary

ATR-FT-IR on the Spectrum Two allows for a fast, easy, and low-cost method for screening olive oil samples for adulterants. The information required from the analysis will determine which will be the most appropriate data analysis method to use. Data has been demonstrated using three different approaches – SIMCA , PLS, and Adulterant Screen. These are summarized below:

SIMCA – Is the product what it says it is and does it fall within the expected variation within that class of material? If not, further data analysis will be required.

PLS - For known adulterants it is possible to generate complete quantitative calibrations by preparing suitable standard mixtures. This will give accurate quantitative results.

Adulterant Screen algorithm – Is the product what it says it is and has it been adulterated? If adulteration is likely then try to identify the adulterant from known adulterants and give a semi-quantitative measure of how much of the adulterant is present.

The Adulterant Screen algorithm offers significant benefits over the other two approaches:

Faster method development

- The Adulterant Screen algorithm simply requires the collection of the spectra of the unadulterated material and the known adulterants.

Simple upgrade of methods

- When new potential adulterants are identified they can simply be added to the library of adulterant spectra.

Greater sensitivity than SIMCA

- Achieved by utilizing a library of spectra of potential adulterants.

Whichever statistical approach is utilized it can be deployed using a Spectrum Touch™ method, employing a simple user interface for the routine operator. Figure 9 is an example of the results screen for an adulterated sample.

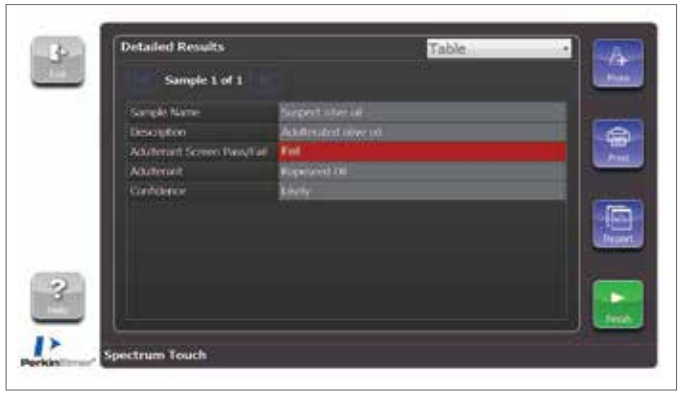

Figure 9. Spectrum Touch software showing result from Adulterant Screen.

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APPLICATION NOTE

Liquid Chromatography

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Analysis of Phenolic Antioxidants in Edible Oil/Shortening Using the PerkinElmer Altus UPLC System with PDA Detection

Introduction

Phenolic antioxidants are commonly used in food to prevent the oxidation of oils. Oxidized oil and fats cause foul odor and rancidity in food products, which is a major cause for concern to the food industry. Globally, regulations vary, but current maximum allowable levels are as low as 100 µg/g (100 ppm).

This application note presents a UHPLC method for the analysis of the ten most common phenolic antioxidants that may be found in such products. The application was carried out with minor modifications to the AOAC Official Method 983.15[1]. This method applies to the analysis of finished food products. A 2.7-µm SPP (superficially porous particle) C18 column was used, allowing one to achieve very high throughput at a back-pressure considerably lower than that for UHPLC columns.

This method was then applied to a commercial vegetable shortening product, which per label claim, was reported to contain at least one of the antioxidants being analyzed.

Method conditions and performance data, including linearity and repeatability, are presented.

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Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer® Altus™ UPLC® System was used, including the Altus A-30 Solvent delivery Module, Sampling Module, A-30h Column Module and PDA (photodiode array) Detector with a 10-mm path-length flow cell. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 Chromatography Data Software (CDS) platform.

Method parameters

The HPLC method parameters are shown in Table 1.

Table 1. UHPLC Method Parameters

HPLC Conditions	
Column:	PerkinElmer Brownlee™ 2.7 µm 2.1 x 100 mm C18 (Part# N9308404)
Mobile Phase:	Solvent A: Water; Solvent B: Acetonitrile Solvent program:

	Time (min)	Flow Rate (mL/min)	0%A	%B	%C	%D	Curve
1	Initial	0.600	60.0	40.0	0.0	0.0	Initial
2	4.50	0.600	45.0	55.0	0.0	0.0	6
3	7.00	0.600	18.0	82.0	0.0	0.0	6
4	10.00	0.600	18.0	82.0	0.0	0.0	6
5	10.10	0.600	60.0	40.0	0.0	0.0	11

Equil. Time ("Next inj. Delay Time"): 3 minutes

Analysis Time:	10 min.
Flow Rate:	0.6 mL/min. (maximum pressure during run: 6600 psi)
Oven Temp.:	35 °C
Detection:	Altus A-30 PDA; wavelength channels: 280 and 220 nm
Injection Volume:	1 µL

Solvents, Standards and Samples All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters.

The phenolic antioxidant standard kit #2 (catalog# 40048-U) was obtained from Supelco® (Irvine, CA). This included nordihydroguaiaretic acid (NDGA), propyl gallate (PG), octyl gallate (OG), lauryl gallate (dodecyl gallate (DG)), 2-tert-butyl-4-hydroxyanisole (BHA), 2,6-di-t-butyl-4-hydroxymethylphenol (Ionox 100), tert-butylhydroquinone (TBHQ), 3,5-di-t-butyl-4-hydroxytoluene (BHT) and ethoxyquin. In addition, a 2,4,5-trihydroxybutyrophenone standard (THBP; catalog# 2620-1-X9) was obtained from SynQuest® (Alachua, FL).

Using a 100-mL volumetric flask, a 100-ppm stock standard was made up by dissolving 10 mg of each of the ten antioxidant standards in methanol and then bringing the flask up to the mark with methanol. Individual calibrant standards were prepared using the 100-ppm stock solution.

The sample ("Sample X") was a commercially available vegetable shortening purchased at a local food market. The sample was prepared by dissolving 3 grams of Sample X in 15 mL of hexane in a 50-mL centrifuge tube and vortexing for 5 minutes. The resulting solution was then extracted with three 30-mL portions of acetonitrile, combining the three extracts into a 250-mL evaporation dish. The combined extract was evaporated down to 1-2 mL and reconstituted to 6 mL with methanol.

Prior to injection, all calibrants and samples were filtered through 0.22-µm filters to remove small particles.

Results and Discussion

Figure 1 shows the chromatographic separation of the 10 phenolic antioxidants in under nine minutes. Figure 2 shows the overlay of 10 replicate 50-ppm standard injections,

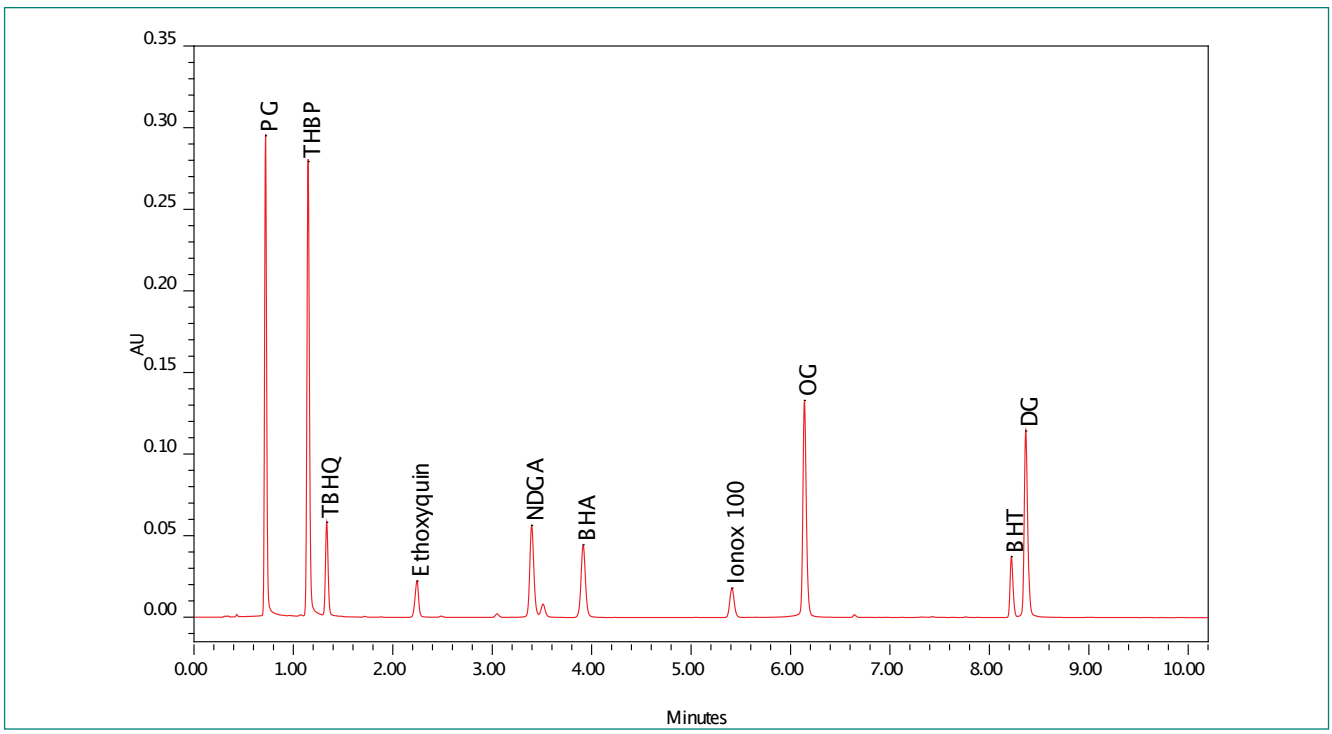

Figure 1. Chromatogram of 50-ppm phenolic antioxidant standard; wavelength = 280 nm.

demonstrating exceptional reproducibility. Retention time % RSDs ranged from 0.10 (early eluters) to 0.03 (later eluters).

In a previous application note [2], it has been noted that ethoxyquin may not be well detected at 280 nm. However, we did not observe this, and we could easily detect the analyte at 5-ppm levels. The same injection was also captured on a separate channel, set to 220 nm, as shown in Figure 3. At this wavelength, it is evident that the ethoxyquin has approximately two times the signal intensity. However, this additional signal intensity was not really required here, as current maximum allowable concentrations for phenolic antioxidants only go down to 100 ppm, which was easily handled at 280 nm.

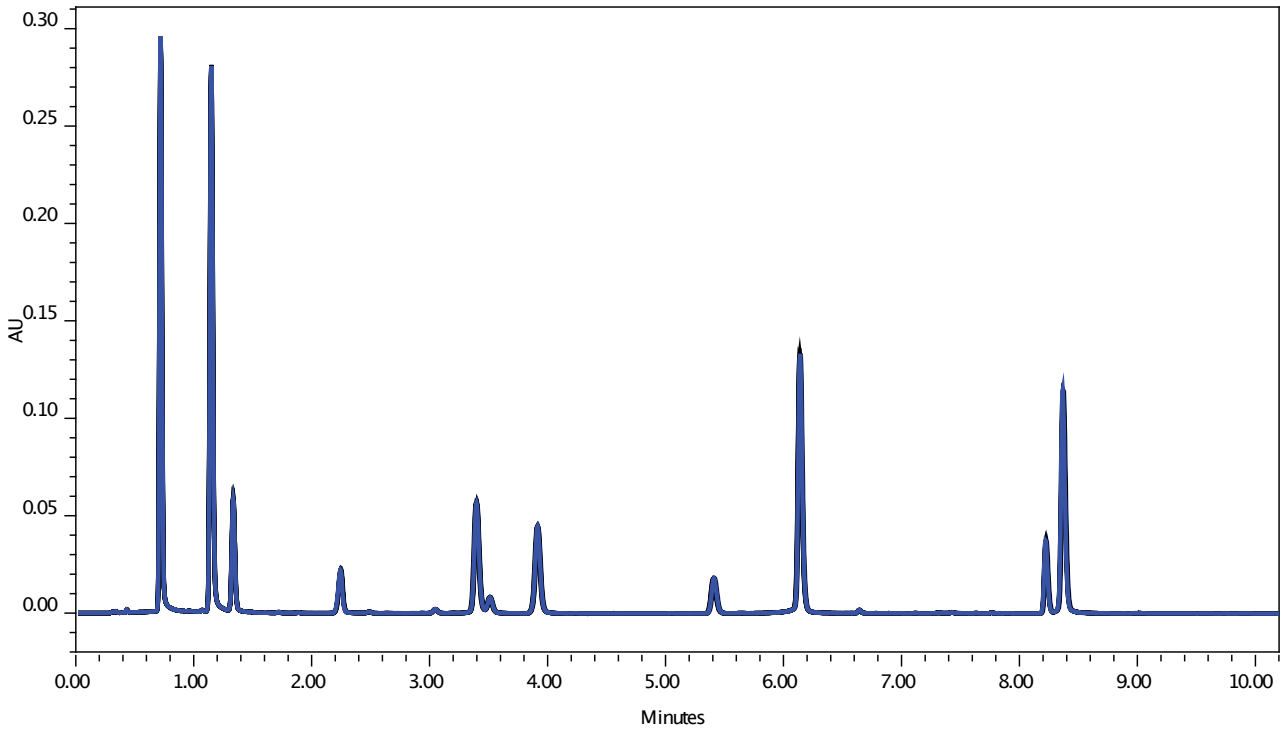

Figure 2. Overlay of 10 replicates of 50-ppm check standard; wavelength = 280 nm.

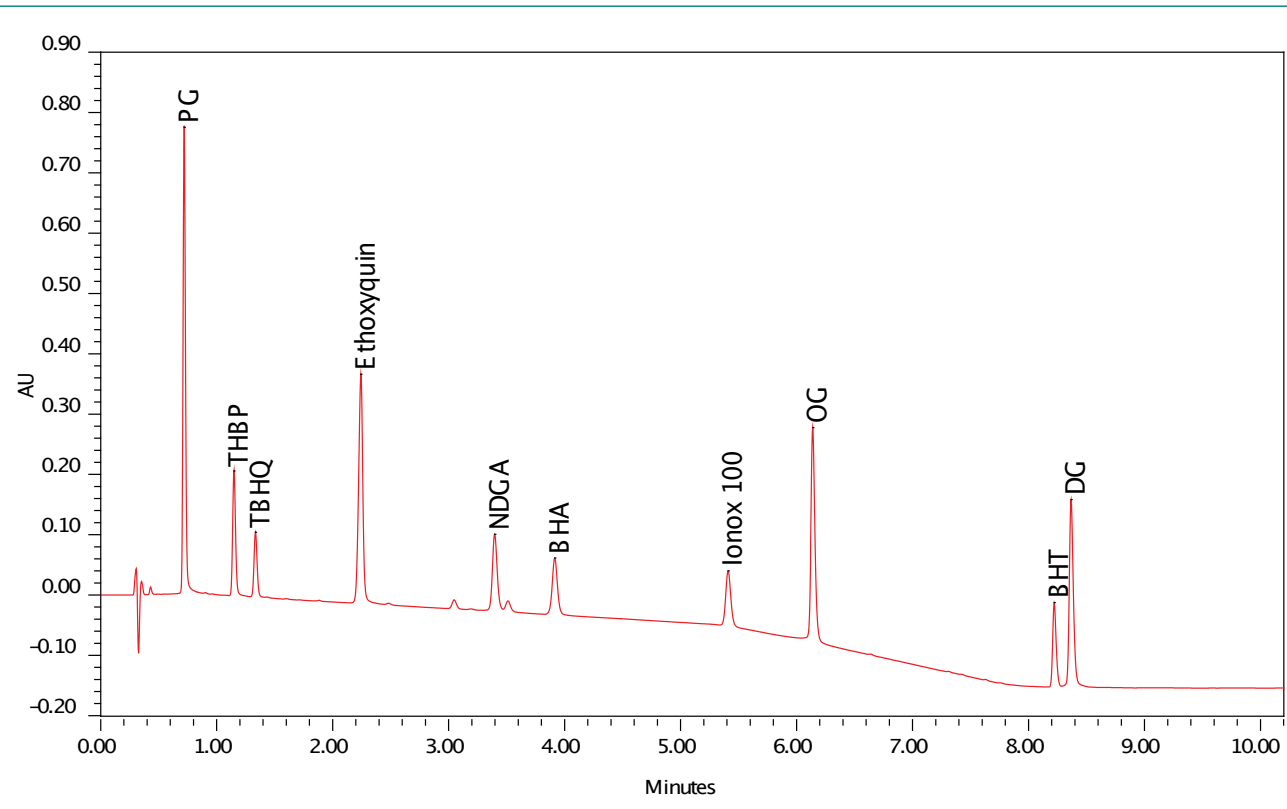

Figure 3. Chromatogram of 50-ppm phenolic antioxidant standard; wavelength = 220 nm.

Figure 4 shows three representative calibration results over a concentration range of 5 to 100 ppm. All ten components had linearity coefficients > 0.999 (n = 3 at each level).

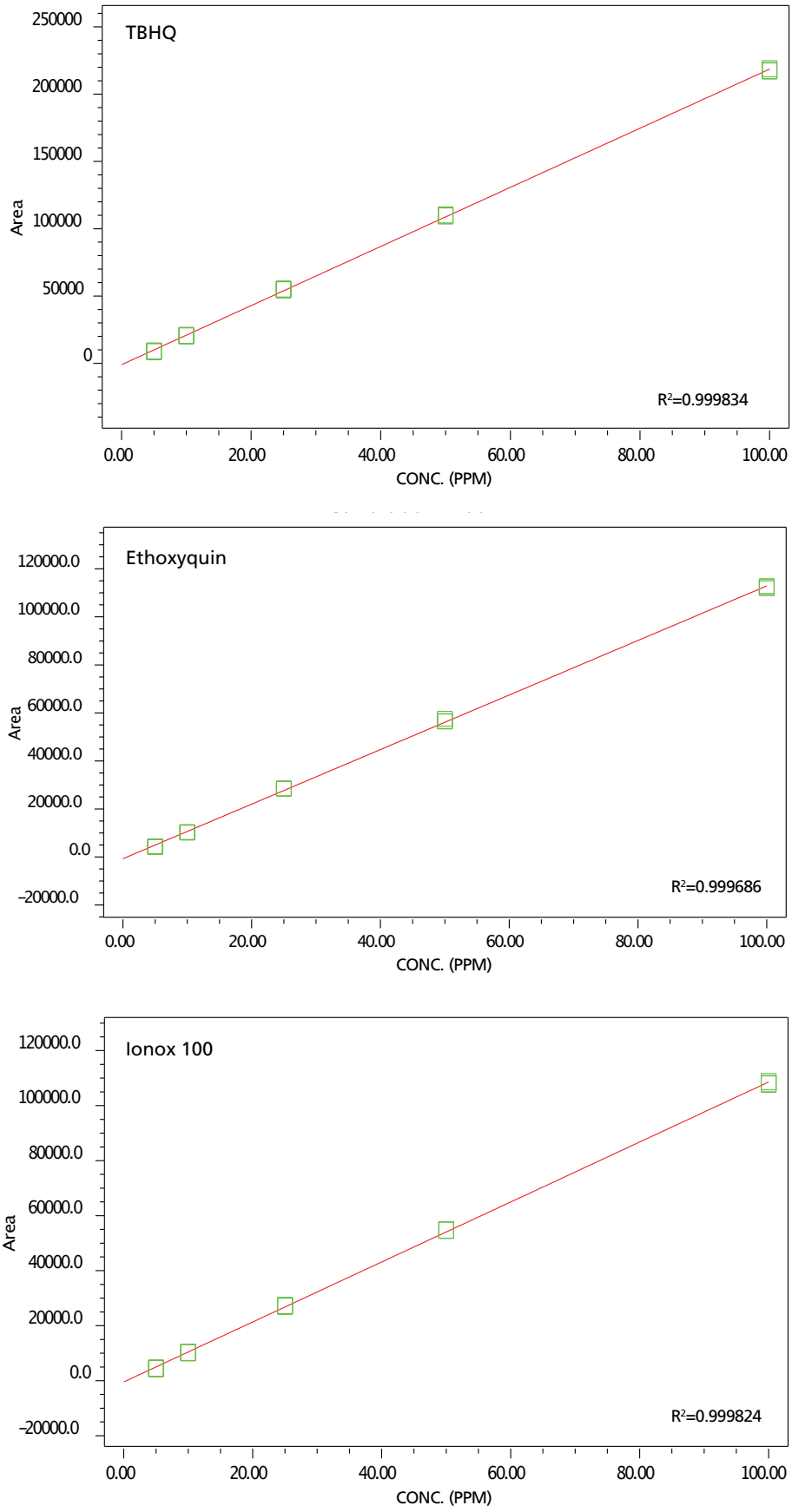

Figure 4. Three representative results of 5-level calibration sets for the phenolic antoxidants; wavelength = 280 nm.

Figure 5 shows the chromatographic results of Sample X overlaid with the 50-ppm standard. A peak eluting at exactly the time of TBHQ (tert-butylhydroquinone) was observed. This was consistent with the product label claim. By back-calculating the concentration in the original sample, it was determined that Sample X contained approximately 12-ppm of TBHQ. The actual concentration could not be verified as it was not provided in the product's label claim.

Per Figure 6, upon closer examination of the chromatogram of Sample X, a small peak at about 8.23 minutes was also observed. This matched the elution time for DG (dodecyl gallate) in the standard mix. If this was indeed DG, its concentration was below the calibration curve, estimated to be <0.5 ppm. Further verification of the identity of this peak was not pursued.

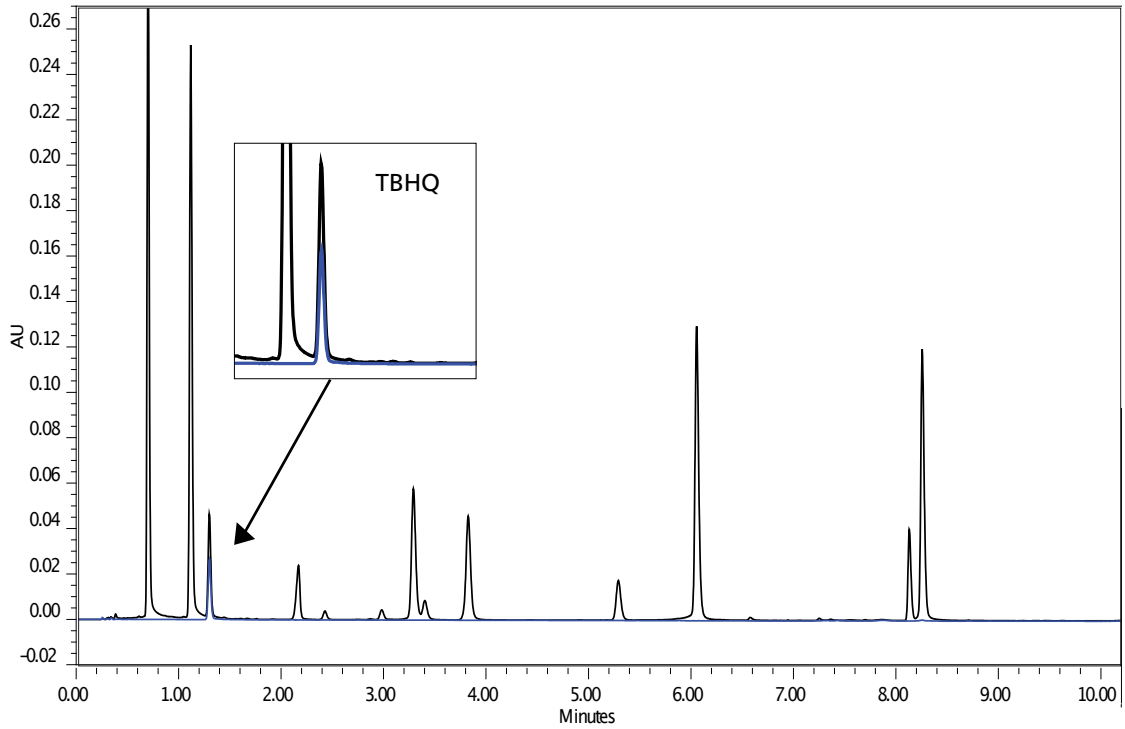

Figure 5. Chromatogram of Sample X (blue) overlaid with 50-ppm standard (black); wavelength = 280 nm.

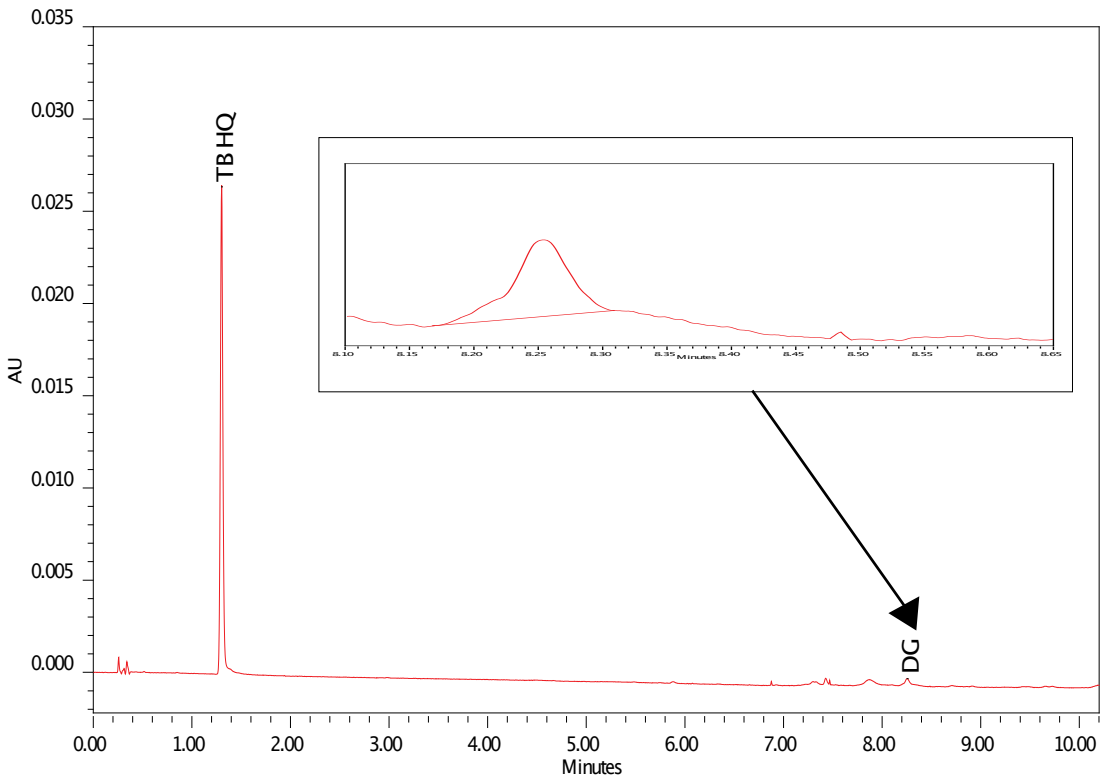

Figure 6. Chromatogram of Sample X with zoomed in area just after 8 minutes; wavelength = 280 nm.

Conclusion

This work has demonstrated the effective chromatographic separation of ten phenolic antioxidants using a PerkinElmer Altus UPLC® with a PDA detector and the Empower® 3 CDS system. The results exhibited excellent retention time repeatability as well as exceptional linearity over the tested concentration ranges. At an analytical wavelength of 280 nm, the sensitivity for all 10 phenolic antioxidants was found to be more than adequate to accommodate the current maximum allowable concentration limit of 100 ppm.

We were able to identify and quantitate the phenolic antioxidant content in a commercial vegetable shortening product and the results matched the label claim of the manufacturer.

From a food quality perspective, considering the ever growing emphasis on food monitoring, this application is intended to serve as a valuable guide for the monitoring of edible oils/shortening. It should be noted that in the U.S., per label claims, only some of the vegetable shortenings reported any amount of phenolic antioxidant. None of the edible oils that were found in stores reported any phenolic antioxidants. However, although only edible vegetable shortening was tested for this study, the provided sample preparation procedure and chromatographic application easily lend themselves to the analysis of edible oils as well.

References

1. Official Methods of Analysis, Method 983.15, Association of Official Analytical Chemists (AOAC), Arlington, VA USA
2. Monitor Antioxidant Additives in Foods, Using HPLC (AOAC Method 983.15) or Capillary GC and a Supelco Reference Standards Kit, Application Note 78, Sigma-Aldrich/Supelco, 2004
3. Analysis of Common Antioxidants in Edible Oil with the PerkinElmer Flexar™ FX-15 System Equipped with a PDA, Application Note, PerkinElmer, Inc.

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APPLICATION NOTE

Liquid Chromatography

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Analysis of Common Antioxidants in Edible Oil with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

Phenolic antioxidants and ascorbyl palmitate (Figure 1, Page 2) are commonly used in food to prevent the oxidation of oils. Oxidized oils cause foul odor and rancidity in food products. This application note will present a UHPLC analysis of edible oils to determine the type and amount of ten different antioxidants.

The method was developed with a 1.9 µm particle size column to achieve very high throughput at a low flow rate, reducing solvent consumption. The throughput of an HPLC method with a 5 µm particle size column will be compared with that of a UHPLC method with a 1.9 µm particle size column. In addition to throughput comparisons, method conditions and performance data, including precision and linearity are presented. The results of the method applied to a spiked oil sample and a sample of vegetable shortening are reported.

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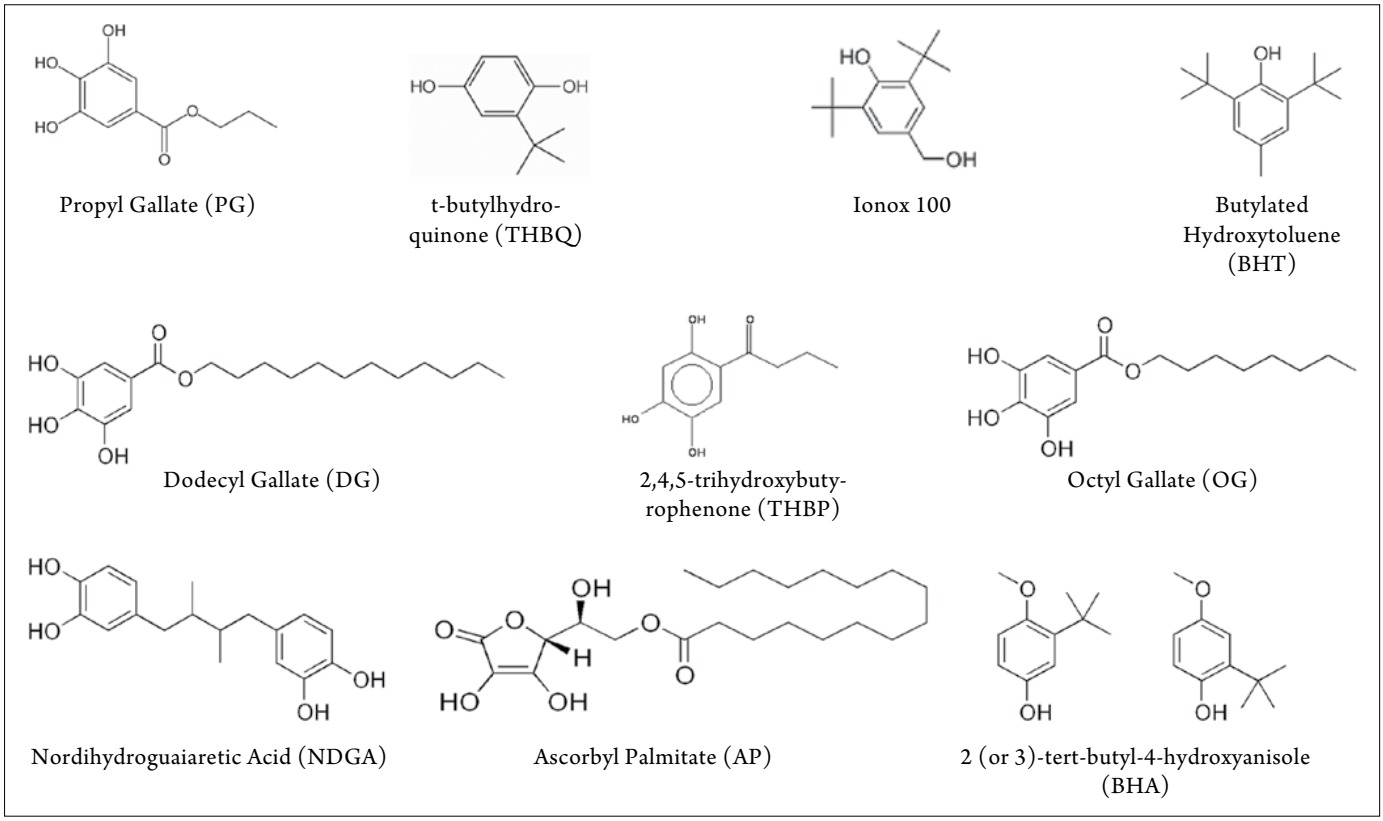

Figure 1. Names and codes of ten antioxidants.

Experimental

The separation was characterized and the system was calibrated with a mixture of antioxidants diluted from neat material. The stock solution contained 0.5 mg/mL of propyl gallate, octyl gallate, dodecyl gallate, nordihydroguaiaretic acid, 2 (or 3)-tert-butyl-4-hydroxyanisole, butylated hydroxy-toluene, 2, 6-di-ter-butyl-4-hydroxymethylphenol (Ionox 100) in methanol; a second stock contained about 0.5 mg/mL of 2,4,5-trihydroxybutyrophenone in methanol; a third stock contained about 0.5 mg/mL of t-butylhydroquinone in methanol; and a fourth stock contained about 0.5 mg/mL of ascorbyl palmitate in methanol with 1 mg/ml of citric acid and 1 mg/mL of isoascorbic acid. The isoascorbic acid and the citric acid as an oxygen quencher and chelating agent were added to methanol to prevent the degradation of ascorbyl palmitate. The working standard with 10 µg/mL of each antioxidant was prepared from the stock standards.

Repeatability was studied with six injections of each standard. Linearity was determined across the range of 0.2 – 10 µg/mL with injections at concentrations: 0.2, 0.5, 1, and 10 µg/mL. Recovery from the sample analysis was tested with canola/olive oil mixture samples spiked with 50 mg/kg of each anti-oxidant and a sample of 0.1 g/mL of vegetable shortening. The samples were diluted with methanol containing 1 mg/mL of citric acid and 1 mg/mL of isoascorbic acid, vortexed for five minutes and centrifuged at 5000 RPM for ten minutes. The supernatants were filtered with a 0.2 µm nylon filter prior to dispensing into UHPLC vials.

A PerkinElmer® Flexar™ FX-15 with Photodiode Array Detector provided the UHPLC platform for this application. The separation was completed on a Restek® Ultra II C18, 1.9 µm 50 mm x 2.1 mm column. The run time was approximately 3.3 min with a back pressure of about 7500 PSI (517 bar).

***Table 1.* Detailed UHPLC System and Chromatographic Conditions.**

Autosampler:	Flexar FX UHPLC
Setting:	50 µL loop and 15 µL needle volume Partial loop mode
Injection:	10 µL conventional C18 HPLC column 2 µL C18 UHPLC column
Detector:	PDA detector 280 nm for phenolics antioxidants and 255 nm for ascorbyl palmitate
Pump:	FX-15
Column:	PerkinElmer C18, 5 µm, 100 x 4.6 mm Restek® Ultra II C18, 1.9 µm, 50 x 2.1 mm Cat # 9604252
Column Temperature:	Ambient, 45° C
Mobile Phase:	B: 70/30 (v/v) acetonitrile/methanol, A: 0.02% formic acid in water (HPLC grade solvent and ACS grade reagent)

Table 1. **Detailed UHPLC System and Chromatographic Conditions,** ***continued.***

Gradient:	**C18 Conventional HPLC column**			
	Time (min)	**Flow (mL/min)**	**B%**	**Curve**
	0.5	1.8	35	1
	2	1.8	35-45	1
	2	1.8	45-100	1
	2.5	1.8	100	1
	C18 UHPLC column			
	Time (min)	**Flow (mL/min)**	**B%**	**Curve**
	0.3	0.7	40	1
	1	0.7	40-75	1
	1	0.7	75-100	1
	1	0.7	100	1
Software:	Chromera® Version 3.0			
Sampling Rate:	5 pts/s			

Results And Discussion

Initially the method was developed with a C18 100 x 4.6 mm conventional HPLC column with 5 µm particle size. The optimal flow rate of this method was determined to be 1.8 mL/min. at ambient temperature. All the antioxidants eluted in seven min (Figure 2). By using a UHPLC shorter column with smaller particle-size (C18 50 x 2.1 mm, 1.9 µm particle size column) the run time was dramatically reduced from 7 min to 3.3 min; the optimal flow rate was 0.7 mL/min and the temperature 45 °C.

The total solvent usage for each injection was 2.3 mL, an impressive improvement from 12.6 mL solvent usage when the conventional HPLC column was used. Thus, more than 80% reduction in solvent usage and more than 50% reduction in testing time was achieved by moving to the UHPLC method. This is important because of the relative high cost of HPLC grade solvent as well as the significant cost of its disposal, resulting in a much lower cost of ownership and a much greener laboratory operation. Representative chromatograms of the standard solution analysis under UHPLC conditions showing the wavelength maximum are presented in Figure 3.

A spectrum of each antioxidant was obtained from the analysis of the standard solution over a range of 190 nm to 700 nm. The spectrum of two antioxidants and an annotated chromatogram of the standard solution under UHPLC conditions are presented in Figures 4 and 5. A representative chromatogram of the spiked canola/olive oil is presented in Figure 6.

Confirming the identity of compounds in the chromatogram of a known or an unknown sample is an important aspect of quality assurance. Confirmation of the presence of TBHQ in the vegetable shortening is demonstrated in Figure 7. In that figure, the spectrum of the peak apex of the highlighted peaks is compared to the spectra of the standard to confirm peak identity.

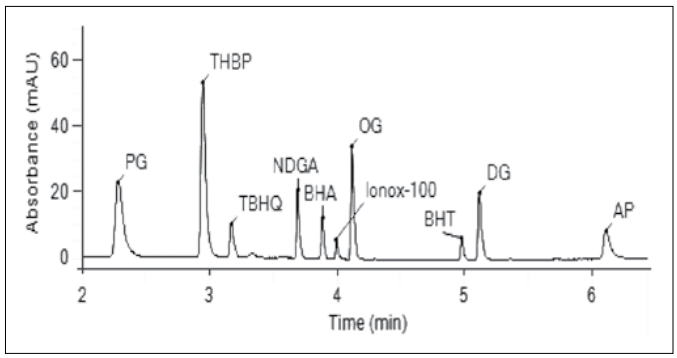

Figure 2. Chromatogram from the analysis standard solution with 10 antioxidants using a C18 HPLC column.

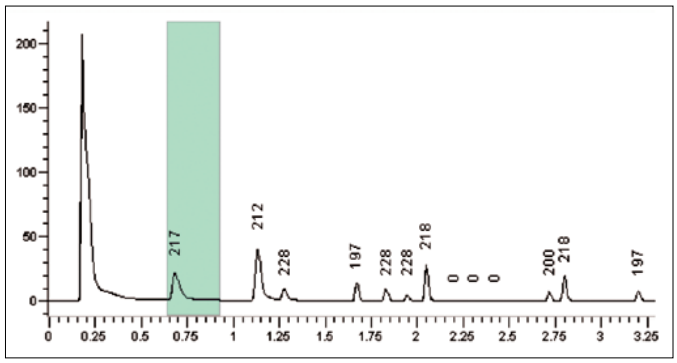

Figure 3. Chromatogram from the analysis of a standard with a C18 UHPLC column showing maximum wavelength absorbance for each peak.

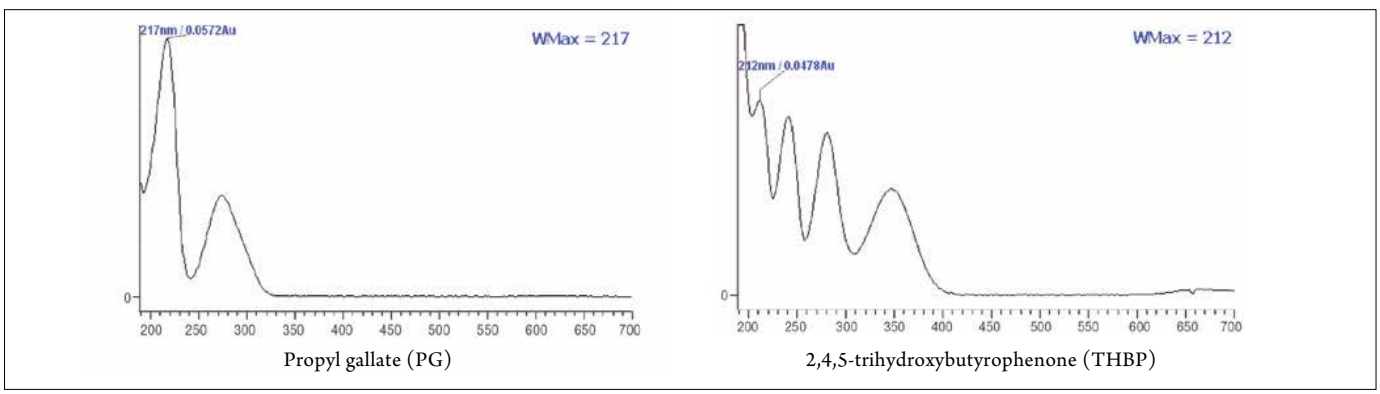

Figure 4. Stored spectra of two antioxidants from the analysis of a standard solution.

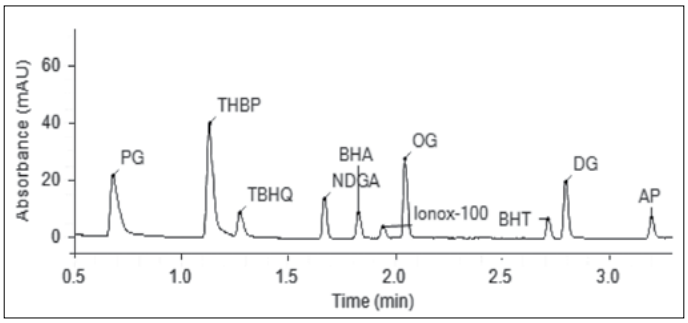

Figure 5. The chromatogram from the analysis standard solution of 10 antioxidants with a C18 UHPLC column.

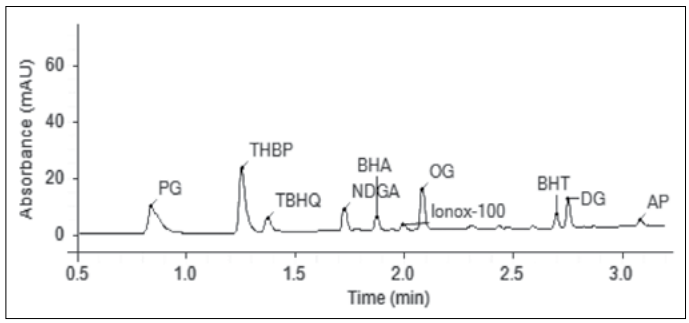

Figure 6. Chromatogram of the analysis of canola/olive oil spiked with 10 antioxidants with a C18 UHPLC column.

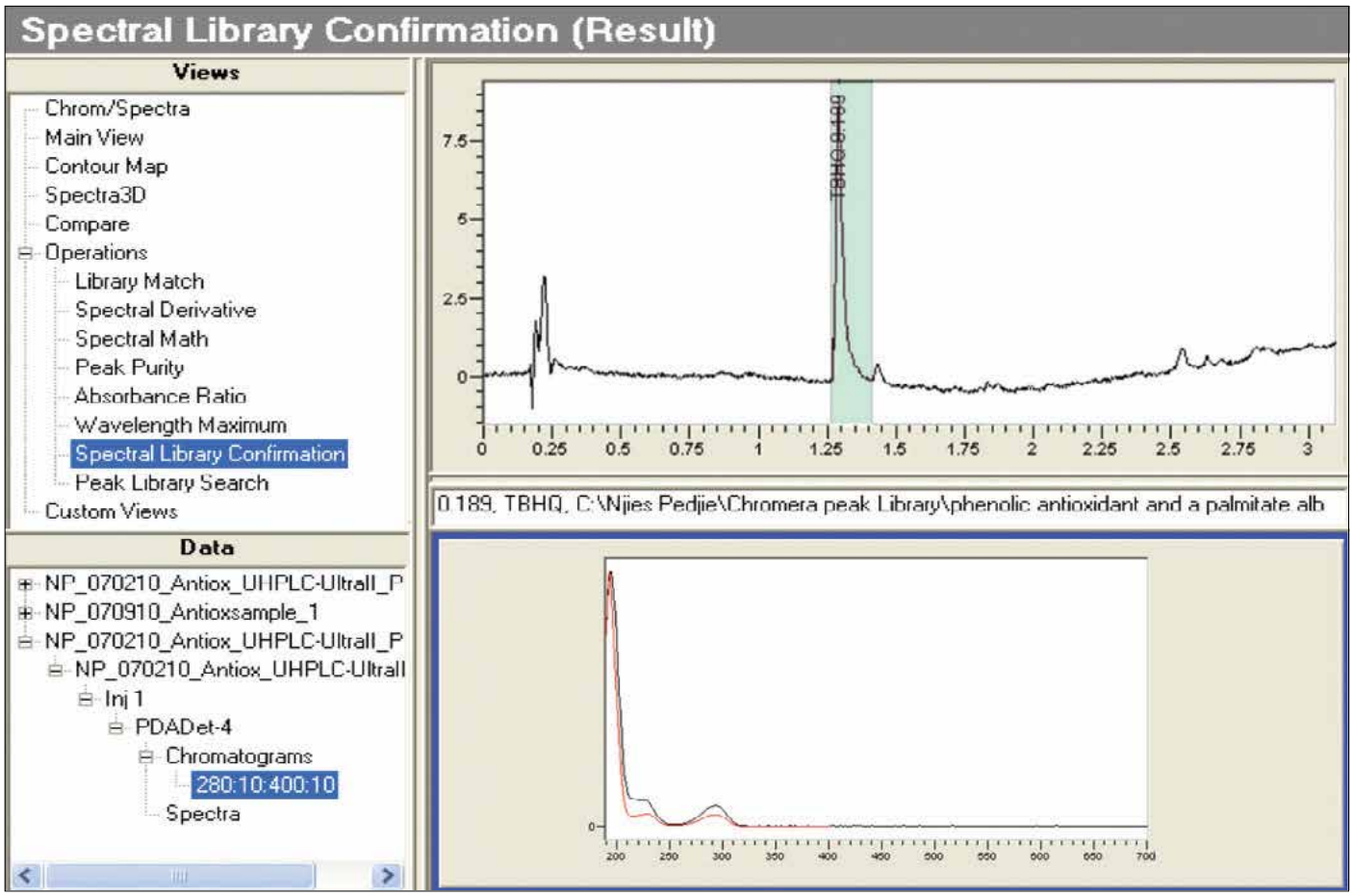

Figure 7. Chromatogram of the analysis of vegetable shortening and the spectra confirmation using a Chromera PDA spectral library.

The method performance was outstanding. The linearity of the analysis achieved an average r^2 value of 0.998. The precision was less than 2.0% relative standard deviation (n=6) for each antioxidant. The canola/olive oil sample resulted in recovery results from 70% and 113%, with an average recovery of approximately 97%. The vegetable shortening tested has 0.01% of TBHQ. Details of the method performance are presented in Table 2.

***Table 2.* Precision, Linearity and Recovery.**

Compound	% RSD (n=6)	Linearity r^2	Canola/olive oil recovery	Vegetable shortening
PG	0.8	0.9992	87%	ND
THBP	1.4	0.9991	99%	ND
TBHQ	1.7	0.9948	96%	0.01%
NDGA	1.1	0.9975	99%	ND
BHA	1.8	0.9991	100%	ND
Ionox-100	1.5	0.9992	96%	ND
OG	1.6	0.9992	103%	ND
BHT	1.3	0.9951	70%	ND
DG	1.5	0.9947	113%	ND
AP	1.1	0.9992	103%	ND

ND = None detected

Conclusion

The application of UHPLC to the analysis of common antioxidants in edible oils has resulted in a 53% reduction in run time as well as an 82% reduction in solvent usage per sample. The PerkinElmer Flexar FX-15 UHPLC and Restek® Ultra II C18, 1.9 µm 50 x 2.1 mm column resolved all antioxidants in about three minutes. All the peaks were well resolved and the method was shown to be linear. The spiked sample recovery was good and the vegetable shortening tested met the TBHQ requirement of not more than 0.02% based on fat content set by the U.S. Code of Federal Regulation. PerkinElmer's PDA provides a rugged and accurate detection over a range of 190 nm to 700 nm encompassing UV and Vis wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, peak purity, spectra 3D and contour maps, which are powerful tools for interrogating the information content of a 3D photodiode array chromatogram. The spectra library search function allowed the storage of the antioxidant spectra that was later used for peak identification and confirmation in the vegetable shortening sample.

References

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2. Federal Register, 21 CFR 172.185.

3. *Bailey's Industrial Oil and Fat Products, Edible Oil and Fat Product,* Vol. 2.

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APPLICATION NOTE

Liquid Chromatography

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Rapid UHPLC Determination of Common Antioxidants in Edible Oils

Introduction

Phenolic antioxidants and ascorbyl palmitate (Figure 1 – Page 2) are commonly used in food to prevent the oxidation of oils. Oxidized oils will cause foul odor and rancidity in food products. This application note will present a UHPLC analysis of edible oils to determine the type and amount of ten different antioxidants.

The method was developed with a 1.9 µm column to achieve very high throughput at a low flow rate, reducing solvent consumption. The throughput of an HPLC method with a 5 µm particle-size column will be compared with that of a UHPLC method and 1.9 µm particle-size column. In addition to throughput comparisons, method conditions and performance data, including precision, linearity, and recovery from spiked samples, will be presented.

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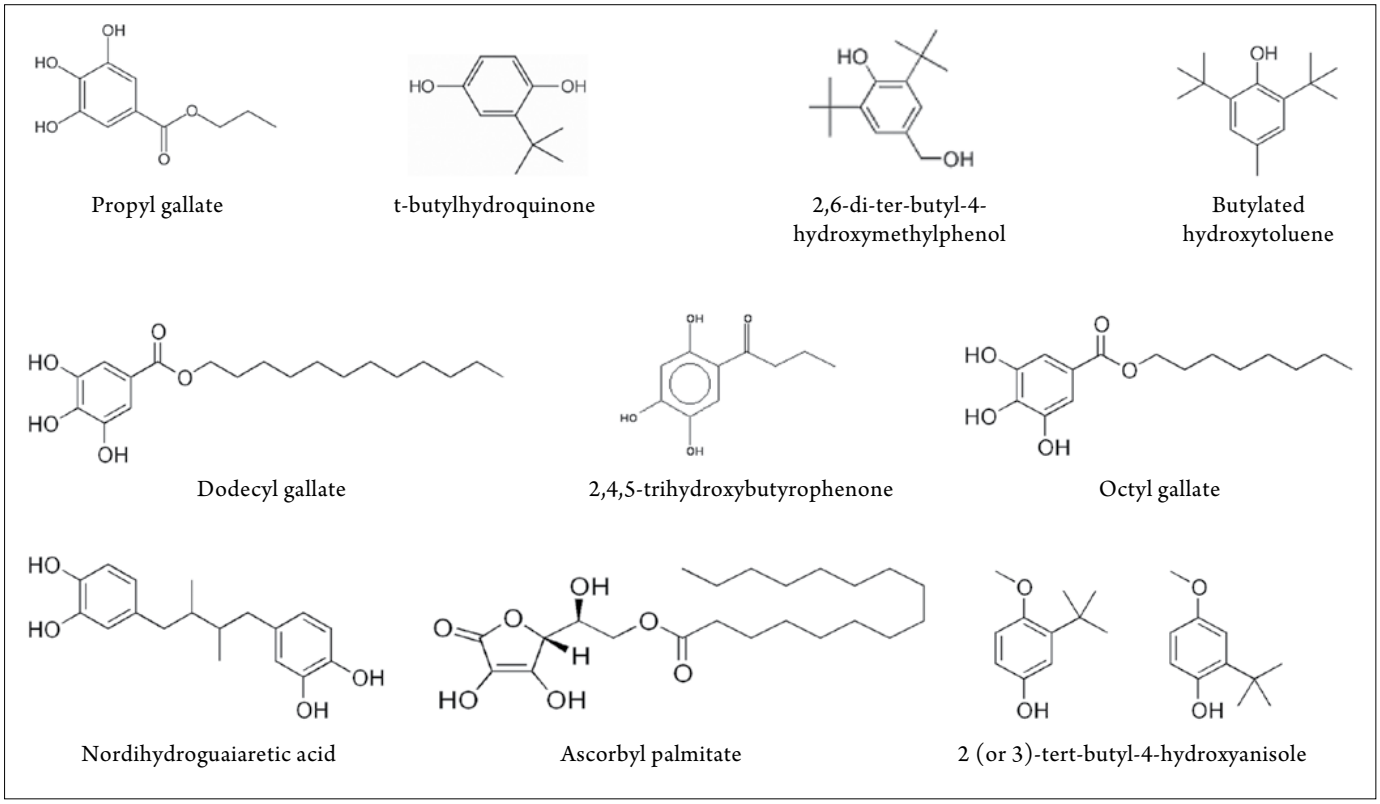

Figure 1. Names and codes of antioxidants.

Experimental

The separation was characterized and system calibrated with a mixture of antioxidants diluted from neat material. One stock solution contained 0.5 mg/mL of propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), nordihydroguaiaretic acid (NDGA), 2 (or 3)-tert-butyl-4-hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 2,6-di-ter-butyl-4-hydroxymethylphenol (Ionox 100) in methanol; a second stock contained about 0.5 mg/mL of 2,4,5-trihydroxybutyrophenone (THBP) in methanol; a third stock contained about 0.5 mg/mL of t-butylhydroquinone (THBQ) in methanol; and a fourth stock contained about 0.5 mg/mL of ascorbyl palmitate (AP) in methanol with 1 mg/mL of citric acid and 1 mg/mL of isoascorbic acid. The isoascorbic acid and the citric acid as an oxygen quencher and chelating agent respectively were added to the methanol to prevent the degradation of ascorbyl palmitate. Working standards with 10 µg/mL of each antioxidant were prepared from the stock standards.

Repeatability was studied with six injections of the working standard. Linearity was determined across the range of 0.2 – 10 µg/mL, with injections at: 0.2, 0.5, 1, and 10 µg/mL. Recovery from sample analysis was tested with oils samples spiked with 50 mg/kg of each antioxidant. A canola-oil and a corn-oil sample were tested. The samples were diluted with methanol containing 1 mg/mL of citric acid and 1 mg/mL of isoascorbic acid, vortexed for 5 minutes and centrifuged at 5000 rpm for 10 minutes. The supernatant were filtered with a 0.2 µm nylon syringe filter prior to dispensing into UHPLC vials.

A PerkinElmer® Flexar™ FX-15 with a Flexar UV/Vis detector provided the UHPLC platform for this application. The separation was completed on a PerkinElmer Brownlee™ Analytical C18, 1.9 µm 50 mm x 2.1 mm column. The run time was approximately 2 min with a back pressure of 8000 psi (552 bar).

Results and Discussion

Initially, the method was developed with phosphoric acid as the modifier in mobile phase A and samples were run using a C18 100 x 4.6 mm, 5 µm particle-size column. The optimal flow rate of this method was determined to be 1.8 mL/min at ambient temperature. All the antioxidants eluted in 7 min. By using a UHPLC shorter column with smaller particle size (C18 50 x 2.1 mm, 1.9 µm particle size), the run time was dramatically reduced from 7 min to about 2 min. The resolution of analyte peaks and sensitivity of the determination were improved by changing the phosphoric acid modifier to formic acid. The optimal flow rate with formic acid was 0.7 mL/min at a temperature of 44 ˚C. An improved separation with sharper peaks and better signal-to-noise characteristics was obtained.

Table 1. **Detailed UHPLC system and chromatographic conditions.**		
Autosampler:	Flexar FX UHPLC, Part No. N2930664	
	Setting: 50 µL loop and 15 µL needle volume, partial loop injection	
	Injection: 10 µL C18 Conventional HPLC column 2 µL C18 HPLC column	
Detector:	Flexar UV/Vis Detector, Part No. N2920013	
	280 nm for phenolics antioxidants and 255 nm for ascorbyl palmitate	
Pump:	Flexar FX-15, Part No. N2910531	
Columns:	PerkinElmer Brownlee Analytical C18, 1.9 µm, 50 x 2.1 mm, Part No. N9303853	
	PerkinElmer C18, 5 µm, 100 x 4.6 mm	
Column temperature:	Ambient, 44 ˚C	
Mobile phase:	B: 70/30 (v/v) acetonitrile/methanol, A: 1% phosphoric acid in water	
	B: 70/30 (v/v) acetonitrile/methanol, A: 0.02% formic acid in water	
	HPLC and ACS® reagent-grade solvents	
Flow rate:	1.8 mL/min C18 Conventional HPLC column	
	0.7 mL/min C18 UHPLC column	
Gradient:	A with phosphoric acid modifier	A with formic acid modifier
	(C18 Conventional HPLC column)	(C18 UHPLC column)
	0.5 min 35% B	0.3 min 38% B
	2 min 35% - 45% B	0.5 min 38% - 70% B
	2 min 45% - 100% B	0.7 min 70% - 100% B
	2.5 min 100% B	0.7 min 100% B
Software:	Chromera® Version 2.1.0.1631	
Sampling rate:	50 pts/s	

The final analysis was completed in 2.2 minutes with a total solvent usage of 1.5 mL for each injection, an impressive improvement from 7 min run time and 12.6 mL solvent usage when the conventional HPLC column was used. Representative chromatograms of standard solution analysis under conventional HPLC and UHPLC conditions are presented in Figures 2 and 3 (Page 4), representative chromatograms of spiked canola oil and corn oil under UHPLC conditions are presented in Figures 4 and 5 (Page 4).

The method performance was outstanding. The linearity of the analysis achieved an average r^2 value of 0.998. The average precision was less than 1% relative standard deviation (n=6). The sample preparation resulted in recovery results between 97% and 114% for both corn and canola oils, with an average recovery of approximately 103%. Details of the method performance are presented in Table 2.

Conclusion

The application of UHPLC to the analysis of common antioxidants in edible oils has resulted in a 4.8 min or about 70% reduction in run time as well as a reduction of solvent usage of 11.1 mL or about 90%. The PerkinElmer Flexar FX-15 UHPLC system and Brownlee Analytical C18, 1.9 µm 50 x 2.1 mm column resolved all antioxidants in about 2 minutes. The method was shown to be linear, the antioxidant peaks were well resolved and the recovery was good.

Reference

1. Perrin C., and Meyer L., *J. Am. Oil Chem, vol 80, no.2 (2003) 115-118.*

Table 2. **Precision, linearity and recovery.**										
	PG	**THBP**	**TBHQ**	**NDGA**	**BHA**	**Ionox-100**	**OG**	**BHT**	**DG**	**AP**
Precision (% RSD)	0.9	0.7	0.9	0.8	1.1	1.0	0.8	1.3	1.0	1.3
Linearity (R^2)	0.9996	0.9999	0.9991	0.9985	0.9990	0.9931	0.9999	0.9944	0.9959	0.9999
Corn oil recovery (%)	107	102	103	102	102	101	101	100	100	105
Canola oil recovery (%)	97	105	105	105	105	104	106	108	106	114

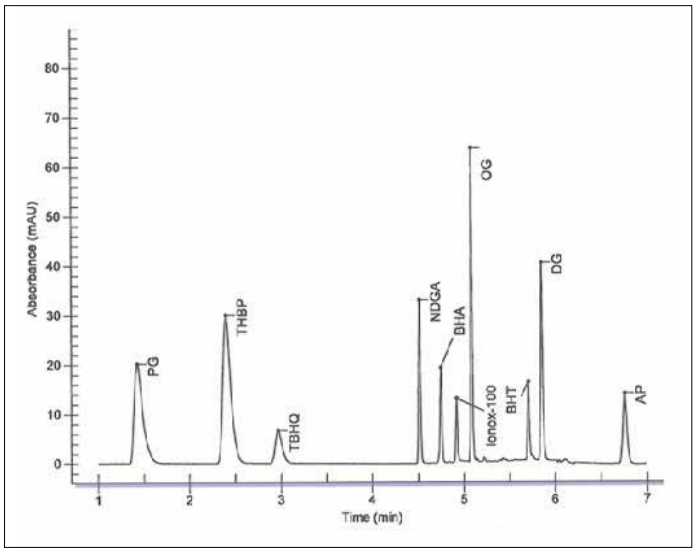

Figure 2. Chromatogram from the analysis of a standard solution with 10 µg/mL of 10 antioxidants using a conventional HPLC C18 100 x 4.6 mm, 5 µm particle-size column.[1]

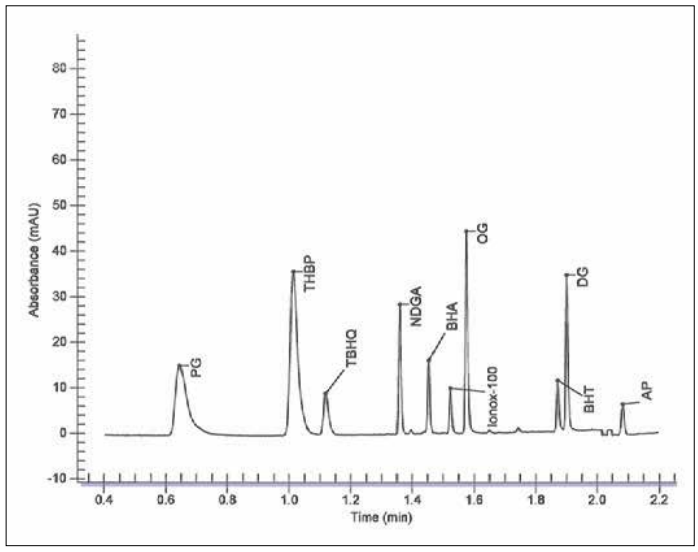

Figure 4. Chromatogram from the analysis of canola oil spiked with common antioxidants.

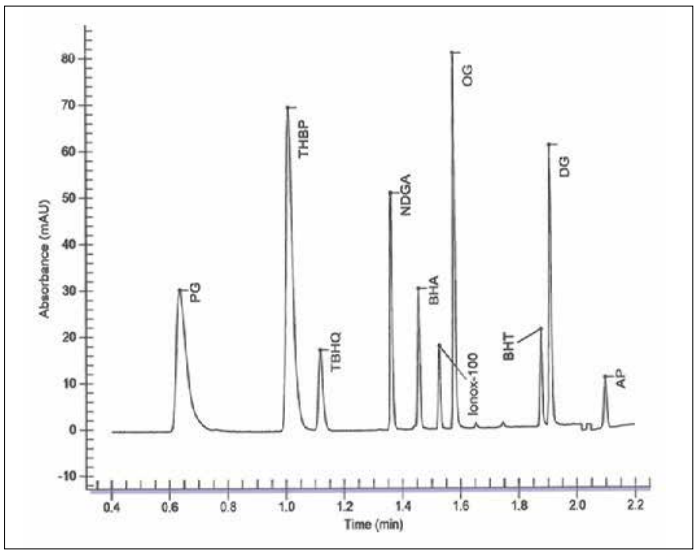

Figure 3. Chromatogram from the analysis of a standard solution with 10 µg/mL of 10 antioxidants using a UHPLC C18 50 x 2.1 mm, 1.9 µm particle-size column.

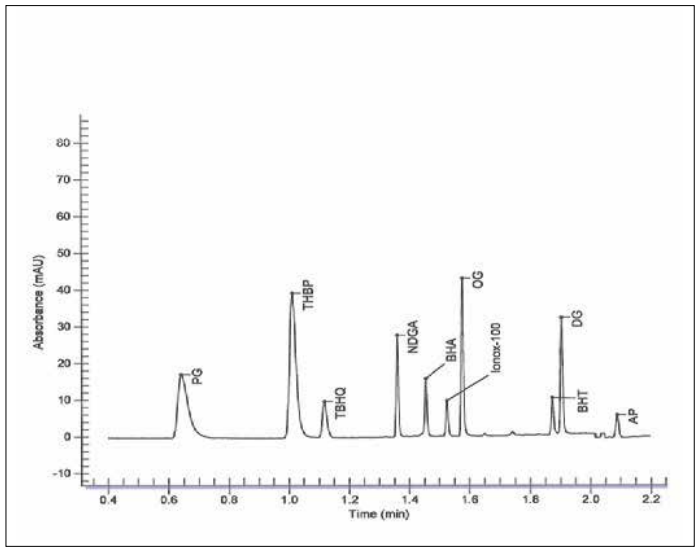

Figure 5. Chromatogram from the analysis of corn oil spiked with common antioxidants.

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APPLICATION NOTE

Atomic Absorption

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Trace Elemental Characterization of Edible Oils with Graphite Furnace Atomic Absorption Spectrophotometer

Introduction

The determination of the inorganic profile of oils is important because of the metabolic role of some elements in the human organism. On the one hand, there is knowledge of the food's nutritional value, which refers to major and minor elements. On the other hand, there is the concern to verify that the food does not contain some minerals in quantities toxic for the health of the consumers, regardless whether this presence of minerals is naturally occurring or is due to contamination during the production processes. Oil characterization is the basis for further nutritional and food technological investigations such as adulteration detection[1]. The most common adulteration is an addition of a cheaper vegetable oil to expensive oil. Authenticity is a very important quality criterion for edible oils and fats, because there is a big difference in prices of different types of oil and fat products. Adulteration detection is possible by determining the ratio of the contents of some chemical constituents and assuming these ratios as constant for particular oil. In regard to adulteration detection, approaches based on atomic spectroscopy can be attractive[2]. The quality of edible oils with regard to freshness, storability and toxicity can be evaluated by the determination of metals. Trace levels of metals like Fe, Cu, Ca, Mg, Co, Ni and Mn are known to increase the rate of oil oxidation. Metals like As, Cd, Cr, Se etc. are known for their toxicities. The development of rapid and accurate analytical methods for trace elements determination in edible oil has been a challenge in quality control and food analysis. However, sample pretreatment procedures are required in order to eliminate the organic matrix. These include wet, dry or microwave digestion, dilution with organic solvent and extraction methods[3]. The content of metals and their species (chemical forms) in edible seed oils depends on several factors. The metals can be incorporated into the oil from the soil or be introduced during the production process. Hydrogenation of edible seed oils and fats has been

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performed using nickel catalysts. The presence of copper and iron can be caused by the processing equipment as well. Different digestion methods were applied for oil digestion prior to spectrometric measurements. Many of the used wet or dry digestion methods are not recommended for use in high fat material because of the associated safety hazards. GFAAS is a suitable and widely used technique for the trace level determination of metals due to its selectivity, simplicity, high sensitivity, and its capability for determination in various matrices.

This paper reports the development of a simple method for the analysis of edible oil samples by using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS). Sample preparation has been done by using a microwave digestion system. Metals like Fe, Cu, Mn, Ni, Cr, As, Cd, Pb, Se and Zn were analyzed using the developed method.

Experimental

The measurements were performed using the PerkinElmer® AAnalyst™ 800 Atomic Absorption Spectrophotometer (PerkinElmer, Inc., Shelton, CT, USA) (Figure 1) equipped with WinLab32™ for AA Version 6.5 software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance. PerkinElmer high efficiency double beam optical system and solid-state detector provide outstanding signal-to-noise ratios. The AAnalyst 800 features longitudinal Zeeman-effect background correction for furnace and the solid-state detector which is highly efficient at low wavelengths. The AAnalyst 800 uses a transversely heated graphite atomizer (THGA) which provides uniform temperature distribution across the entire length of the graphite tube. This eliminates the memory effect inherent with the high matrix sample analysis. The THGA features an integrated L'vov platform which is useful in overcoming potential chemical interference effects common to the GFAAS technique. EDL lamps were used whenever available.

Figure 1. PerkinElmer AAnalyst 800 Atomic Absorption Spectrophotometer.

A Multiwave™ 3000 Microwave system (PerkinElmer/Anton-Paar) was used for the microwave-assisted digestion. This is an industrial-type oven which is equipped with various accessories to optimize the sample digestion.

The samples were digested in the Rotor 8XF100 in eight 100 mL high pressure vessels made of PTFE-TFM protected with its individual ceramic jackets. TFM is chemically modified PTFE that has enhanced mechanical properties at high temperatures compare to conventional PTFE. This vessel has a "working" pressure of 60 bar (870 psi) and temperatures of up to 260 ˚C. A Pressure/Temperature (P/T) Sensor Accessory was also used for this work. The P/T sensor simultaneously measures temperature and pressure for one vessel. All vessels' temperatures were monitored with the IR Temperature Sensor Accessory. This measures the temperature of the bottom surface of each vessel liner remotely during the digestion process, thus providing the over-temperature protection to each vessel.

Figure 2. PerkinElmer/Anton Paar Multiwave 3000 Microwave Digestion System.

Standards, Chemicals and Certified Reference Material

PerkinElmer single element calibration standards for Atomic Spectroscopy were used as the stock standards for preparing working standards. All the working standards were prepared daily in Millipore® water (18.2 MΩ cm) acidified in 0.2% Suprapur® nitric acid. Suprapur® nitric acid used for preparing the diluent for standards was from Merck® (Darmstadt, Germany). Chemical modifiers were prepared from stock solutions, by diluting with acidified Millipore® water and were added automatically to each standard, blank and sample by the autosampler AS 800, an integral part of the AAnalyst 800. Standards were prepared in polypropylene vials (Sarstedt®) and were prepared on volume-by-volume dilution. Micropipettes (Eppendorf®, Germany) with disposable tips were used for pippetting solutions. Certified Reference Standard for trace metals in soybean oil from High Purity Standards (Lot # 0827322) was used for quality control. Multi-element ICP standard for trace metal ions in nitric acid from Spex Certiprep®. (New Jersey, USA), prepared at midpoint of the calibration curve was used as quality control check standard.

Sample Preparation

Three common edible oils: coconut oil, sunflower oil and soybean oil were bought from a local supermarket and were used without any pre-treatments. ~0.25 g of each sample, accurately weighed in duplicate was transferred to the digestion vessels of the microwave digestion system and the sample digestion was done in accordance with the program given in Table 3.

The digested samples were diluted with 0.2% HNO_3 and made up to 25 mL in polypropylene vials. Plastic bottles were cleaned by soaking in 10% (v/v) HNO_3 for at least 24 hours and rinsed abundantly in de-ionized water before use.

The instrumental conditions for furnace experiments are given in Table 1, and the graphite furnace temperature programs are listed in Appendix I. A heated injection at 90 °C was used for all the experiments. Pyrolytically coated graphite tubes with integrated platforms were used. The autosampler cups were soaked in 20% nitric acid overnight to minimize sample contamination, and thoroughly rinsed with 0.5% HNO_3 acid before use. Five point calibration curves (four standards and one blank) were constructed for all the metal ions and the calibration curve correlation coefficient was ensured to be better than 0.999 before the start of the sample analysis.

Dilution of the order of 1000-10000 were required depending up on the metal ion analyzed.

Results and Discussion

The goal of this method development was to develop a simple method for the quantitative analysis of various toxic metals and other trace metals in edible oils. The validity of the developed method has been ensured by incorporating various Quality Control (QC) checks and analysis of Certified Reference Material (CRM).

The QC samples gave excellent recovery of between 94-111%. Excellent spike recovery for these QC samples ranging between 91-109% was also achieved. The recovery for the reference soybean oil was between 91-107%.

Method detection limits (MDLs) were calculated based on the standard deviation of seven replicates of the samples (student t-value of 3.14 for a confidence interval of 98%).

The excellent detection limits obtained shows the capability of AAnalyst 800 in analyzing difficult matrices at lower concentrations. The calibration curves obtained had correlation coefficient as good as 0.999 for all the metal ions under study.

Conclusions

A simple method for the sequential quantitative determination of trace metal impurities in edible oil samples was developed. The patented THGA tube used in the AAnalyst 800 provides a uniform temperature distribution along its entire length. This eliminates cooler temperatures at the tube ends and removes most interference. There is no re-condensation, carry-over and memory effect is eliminated. With the THGA tube design, accuracy and sample throughput are improved by reducing the need for the time-consuming standard additions technique. With the longitudinal Zeeman-effect background correction, the amount of light throughput is doubled by eliminating the need for a polarizer in the optical system. All other commercial Zeeman designs incorporate inefficient polarizers that reduce light throughput and diminish performance. With this unique design, the AAnalyst 800 provides the lowest detection limits available.

In conventional furnace systems, the heating rate during atomization depends on the input-line voltage. As voltage may vary from day to day, season to season or among laboratory locations, so may the heating rate. The high-performance AAnalyst 800 uses enhanced power control circuitry to maintain a uniform heating rate, irrespective of the location of the instrument, one can be sure that it provides outstanding, and consistent performance.

The AAnalyst 800 atomic absorption spectrophotometer also produces highly accurate, fast and reproducible results with difficult matrices such as edible oil. The developed method has been validated by using reference material and the method has been successfully applied for the analysis of different edible oil samples.

The Multiwave 3000 microwave digestion system has proven to be an excellent tool for digesting difficult matrices such as edible oils.

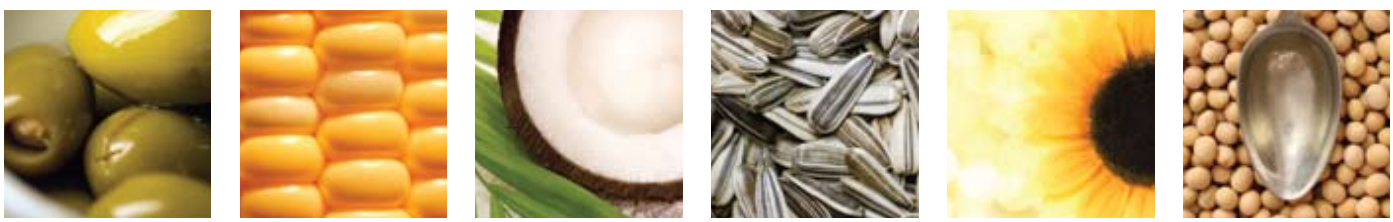


Table 1. **Experimental Conditions of AAnalyst 800.**

Element	Cd	Pb	As	Se	Ni	Cu	Fe	Mn	Cr	Zn
Wavelength (nm)	228.8	283.3	193.7	196	232	324.8	248.3	279.5	357.9	213.9
Slit (nm)	0.7	0.7	0.7	2.0	0.2	0.7	0.2	0.2	0.7	0.7
Mode	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG
Calibration	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.
Lamp	EDL	EDL	EDL	EDL	HCL	HCL	HCL	HCL	HCL	HCL
Current (mA)	230	440	380	280	25	15	30	20	25	15
Standards (µg/L)	0.5, 1.0 1.5, 2.0	20, 30 40, 50	20, 30 40, 50	25, 50 75, 100	20, 30 40, 50	5, 10 15, 25	5, 10 15, 20	2.5, 5 7.5, 10	4, 6 8, 10	0.4, 0.8 1, 2
Correlation coefficient	0.999831	0.999799	0.999656	0.999789	0.999735	0.999533	0.999035	0.999581	0.999707	0.999972
Spike (µg/L)	1.0	10	10	10	5	2.5	5	1.5	4	0.5
Read time (sec)	5	5	5	5	5	5	5	5	5	5
Measurement	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area
Injection Temp (˚C)	90	90	90	90	90	90	90	90	90	90
Sample Volume µL	20	20	20	20	20	20	20	20	20	20
Matrix modifier	0.05 mg $NH_4H_2PO_4$ & 0.003 mg $MgNO_3$	0.05 mg $NH_4H_2PO_4$ & 0.003 mg $MgNO_3$	0.005 mg Pd and 0.003 mg $MgNO_3$	0.005 mg Pd and 0.003 mg $MgNO_3$	Nil	0.005 mg Pd and 0.003 mg $MgNO_3$	0.015 mg $MgNO_3$	0.005 mg Pd and 0.003 mg $MgNO_3$	0.015 mg $MgNO_3$	0.005 mg $MgNO_3$
Modifier volume µL	5	5	5	5	0	5	5	5	5	5

Table 2. **Program used for Edible Oil Digestion with MDS.**

Sequence	Power	Ramp Time (min.)	Hold Time (min.)	Fan
1	600	5	2	1
2	900	5	2	1
3	1400	15	20	1
4	0	0	15	3
Weight Taken	~250 mg	HNO_3	5 mL	
H_2O_2	3 mL	Rate	0.3 bar/sec	
HCl	1 mL	Pressure	55 Bar	

Table 3. **Results of Edible Oil Analysis.**

Metal	Coconut Oil I µg/g	Coconut Oil II µg/g	Sunflower Oil I µg/g	Sunflower Oil II µg/g	Soybean Oil I µg/g	Soybean Oil II µg/g
Pb	<DL	<DL	<DL	<DL	<DL	<DL
Cd	<DL	<DL	<DL	<DL	<DL	<DL
Cr	0.40	0.43	1.1	1.4	0.39	0.42
As	<DL	<DL	<DL	<DL	<DL	<DL
Se	0.08	0.12	0.10	0.07	0.05	0.03
Zn	0.78	0.79	0.80	1.5	3.9	2.26
Cu	0.12	0.11	0.14	0.13	0.09	0.10
Mn	0.57	0.60	0.21	0.19	0.15	0.18
Ni	0.24	0.23	4.16	5.27	0.37	0.25
Fe	7.05	6.75	3.93	4.3	4.08	4.6

Table 4. **Results of CRM Analysis (Lot # 0827322).**

Metal	Certified Value (µg/g)	% Recovery
Pb	100 ±1.0	100.3
Cd	Not present	–
Cr	Not present	–
As	Not present	–
Se	Not present	–
Zn	100 ±1.0	94.0
Cu	100 ±1.0	96.1
Mn	Not present	–
Fe	100 ±1.0	91.1
Ni	100 ±1.0	107.3

Table 5. **Results of QC and Spike Recovery.**

Metal	QC 1 (%)	QC 2 (%)	Spike Recovery (%)
Pb	104.6	102.9	99.9
Cd	102.0	105.6	100.1
Cr	101.8	99.0	101.6
As	100.6	103.1	109.4
Se	104.3	102.5	97.7
Zn	97.3	97.3	111
Cu	96.0	98.7	93.5
Mn	100.7	104.6	91.2
Fe	96.1	94.6	102.4
Ni	109.1	101.8	101.8

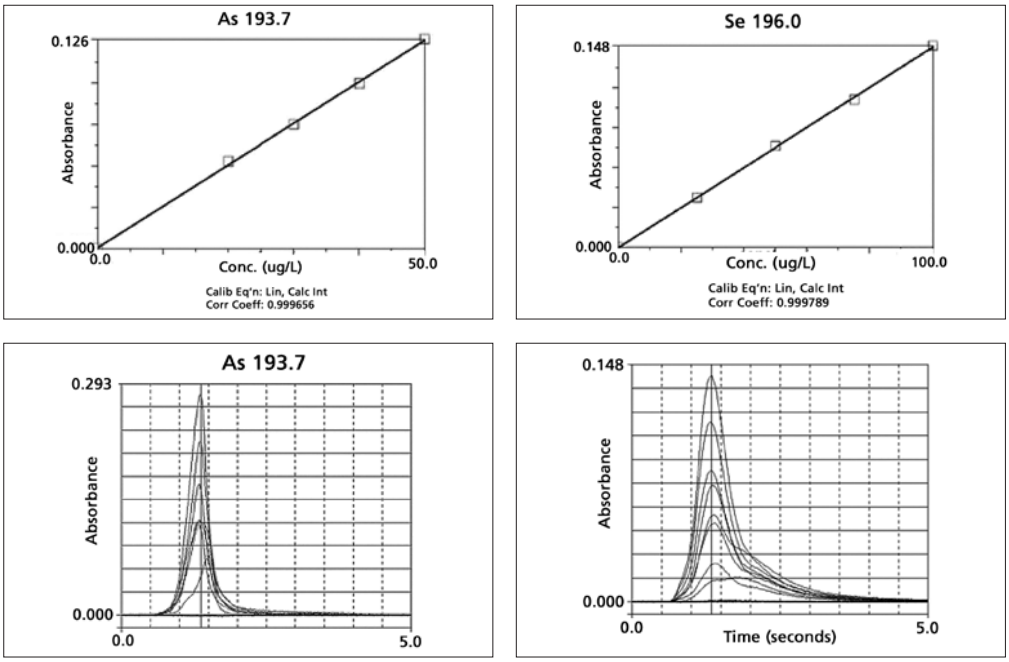
Table 6. **Method Detection Limits (MDLs).**

Metal	MDL (µg/kg)
Pb	19.8
Cd	0.8
As	48.4
Se	167.9

Appendix I. **Graphite Furnace Temperature Program.**

Element	Step	Temp ˚C	Ramp Time (Sec)	Hold Time (Sec)	Internal Gas Flow (mL/min)	Gas Type
Se	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1300	10	20	250	Argon
	4	1900	0	5	0	Argon
	5	2450	1	3	250	Argon
Cd	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	500	10	20	250	Argon
	4	1500	0	5	0	Argon
	5	2450	1	3	250	Argon
As	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1200	10	20	250	Argon
	4	2000	0	5	0	Argon
	5	2450	1	3	250	Argon
Cu	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1200	10	20	250	Argon
	4	2000	0	5	0	Argon
	5	2450	1	3	250	Argon
Ni	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1100	10	20	250	Argon
	4	2300	0	5	0	Argon
	5	2450	1	3	250	Argon
Fe	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1400	10	20	250	Argon
	4	2100	0	5	0	Argon
	5	2450	1	3	250	Argon
Pb	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	850	10	20	250	Argon
	4	1600	0	5	0	Argon
	5	2450	1	3	250	Argon
Zn	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	700	10	20	250	Argon
	4	1800	0	5	0	Argon
	5	2450	1	3	250	Argon
Cr	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1500	10	20	250	Argon
	4	2300	0	5	0	Argon
	5	2450	1	3	250	Argon
Mn	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1300	10	20	250	Argon
	4	1900	0	5	0	Argon
	5	2450	1	3	250	Argon

Appendix II. **Examples of Typical Calibration Curves and Atomization Profiles.**



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APPLICATION NOTE

Atomic Absorption

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Toxic Trace Metals in Edible Oils by Graphite Furnace Atomic Absorption Spectrophotometry

Introduction

Graphite furnace atomic absorption spectrophotometry (GFAAS) has been widely applied to the determination of trace elements in food due to its selectivity, simplicity, high sensitivity, and its capability for accurate determinations in a wide variety of matrices. Edible oils are generally low in trace element concentrations, however, metals such as arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), and selenium (Se) can be found and are known for their toxicities which affect the health of consumers. The determination of toxic elements from naturally occurring or production-contamination sources in oils can be determined by using GFAAS or inductively coupled plasma mass spectrometry (ICP-MS). When only a few elements are being analyzed, GFAAS is the preferred choice. It is easy to learn, faster in setting up, and simpler to use than ICP-MS. GFAAS is also lower in initial capital investment and has a lower operating and maintenance cost. Sample pretreatment procedures for edible oils are normally required prior to instrumental analysis in order to eliminate the organic matrix. Wet, dry or microwave digestion, dilution with organic solvent, and extraction methods can be time consuming and require more operator training than a direct analysis method.

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This method reports the development of a direct analysis method for edible oil samples using GFAAS without digestion. The advantages of using this method include small sample volume, direct introduction of samples, high sensitivity, and rapid analysis times. The application of GFAAS to arsenic, lead and cadmium analysis in edible oils was performed. The optimal pyrolysis and atomization temperatures, limit of detection, quality control (QC) checks and recoveries were studied in order to develop a rapid and accurate method.

Experimental Conditions

Instrumentation

The measurements were performed using the PerkinElmer PinAAcle™ 900T atomic absorption spectrophotometer (PerkinElmer, Inc., Shelton, CT, USA) (Figure 1) equipped with an AS 900 graphite furnace autosampler and WinLab32™ for AA software running under Microsoft® Windows™ 7 operating system.

Figure 1. PerkinElmer PinAAcle 900T atomic absorption spectrophotometer equipped with AS 900 graphite furnace autosampler.

The PerkinElmer PinAAcle 900T has a high-efficiency, true double-beam optical system and solid-state detector which provide outstanding signal-to-noise ratios. The system features longitudinal Zeeman-effect background correction for the graphite furnace, doubling the amount of light throughput by eliminating the need for a polarizer in the optical system. The use of standard transversely heated graphite atomizer (THGA) tubes provides uniform temperature distribution across the entire length of the graphite tube. By employing the latest analytical concepts of the Stabilized Temperature Platform Furnace™ (STPF) technique and the instrumental advances of THGA tubes, chemical interferences are overcome allowing for faster, simpler direct calibration.

The analytical conditions (Table 1) and the graphite furnace temperature programs (Table 2) are given below. A heated injection at 90 ˚C was used for all three elements. Standard (non-endcapped) pyrolytically coated THGA tubes (Part No. B3000641) were used for all analyses. The autosampler cups were soaked in 20% nitric acid overnight to minimize sample contamination, and were thoroughly rinsed with deionized water before use. Prior to unknown sample analysis, a five-point calibration curve (four standards and one blank) using isopropyl alcohol (IPA) was constructed for each analyte. By employing the latest analytical concepts of the Stabilized Temperature Platform Furnace (STPF) technique and the instrumental advances of THGA tubes, chemical interferences are overcome allowing for faster, simpler direct calibration.

Table 1. **Analytical conditions for analyzing several toxic metals in edible oils on the PinAAcle 900T.**

Analyte	As	Pb	Cd
Wavelength (nm)	193.70	283.31	228.80
Slit Width (nm)	0.7	0.7	0.7
Lamp Type	EDL	EDL	HCL
Signal Processing	Peak Area	Peak Area	Peak Area
Read Time (sec)	3	3	2
Standard/Sample Volume (µL)	20	20	20
Diluent Volume (µL)	4	4	5
Matrix Modifier	5 µg Pd + 0.5 µg Mg	5 µg Pd + 0.5 µg Mg	5 µg Pd + 0.5 µg Mg
Matrix Modifier Volume (µL)	5	5	5
Injection Temp (˚C)	90	90	90
Pipet Speed (%)	40	40	40
Calibration Equation	Linear Through Zero	Linear Through Zero	Linear Through Zero
Standard Concentration (µg/L)	0, 20, 30, 40, 50	0, 20, 30, 40, 50	0, 0.5, 1.0, 1.5, 2.0
QC Concentration (µg/L)	10	10	0.4
Automatic Spike Conc. (µg/L)	10	10	0.5

Table 2. **Temperature programs for analyzing several toxic metals in edible oils on the PinAAcle 900T.**

Analyte	As			Pb			Cd		
Furnace Program	**Temp (˚C)**	**Ramp (s)**	**Hold (s)**	**Temp (˚C)**	**Ramp (s)**	**Hold (s)**	**Temp (˚C)**	**Ramp (s)**	**Hold (s)**
Drying 1	110	1	20	110	1	20	110	1	20
Drying 2	150	10	10	150	10	10	150	10	10
Drying 3	450	10	20	450	10	20	450	10	20
Pyrolysis	1100	10	20	900	10	20	550	10	20
Atomization	2300	0	3	1900	0	3	1800	0	2
Clean Out	2500	1	5	2500	1	5	2500	1	5

BOC = 2 s for all samples

Standards and Sample Preparation

Single-element PerkinElmer Pure Calibration Standards (Part Nos. As: N9300180; Pb: N9300175; Cd: N9300176) were used as the stock standards for preparing working standards and quality control check standards. All standards were prepared with IPA (VWR, Normapur Reagent grade) by volume/volume (v/v) dilution.

A mixture of 1000 mg/L Pd and 100 mg/L Mg was used as the chemical modifier for all elements. The chemical modifier was prepared by weighing 0.1430 g of Pd(II) acetylacetonate (Aldrich, 99%, MW=304.62) and pipetting 1 mL of Mg oil standard (Conostan, 5000 µg/mL) and dissolving with 50 mL of xylene (Panreac, reagent grade).

Five edible oils (palm, sesame, sunflower, soybean and rice bran) were purchased from a local supermarket and were used without any pre-treatment. All oil samples were carefully diluted 20 times (v/v) with isopropyl alcohol in polypropylene vials (Part No. B0193234).

Results and Discussion

The calibration curves for all elements returned an r^2 value ≥ 0.997 (Figure 2). Direct calibration for the analysis of oil samples has several advantages over the method of standard additions. Direct calibration results in less operator error, lower cost, and shorter analysis times than with standard additions or matrix matched standards.

An overlay of the peak plots for the standards (red), QC checks (green), and oil samples (various colors) taken on the PinAAcle 900T spectrometer are shown in Figure 3 (Page 4). Although there is a difference in appearance times for some elements, when using the conditions listed above and calculating for peak area, the results are accurate and precise.

The results for the direct analysis of edible oils using GFAAS to detect toxic metals are shown in Table 3 (Page 4). All oils showed concentrations less than the detection limit for lead and cadmium. The soybean oil showed a concentration of 4.28 µg/L arsenic. All others showed concentrations less than the detection limit.

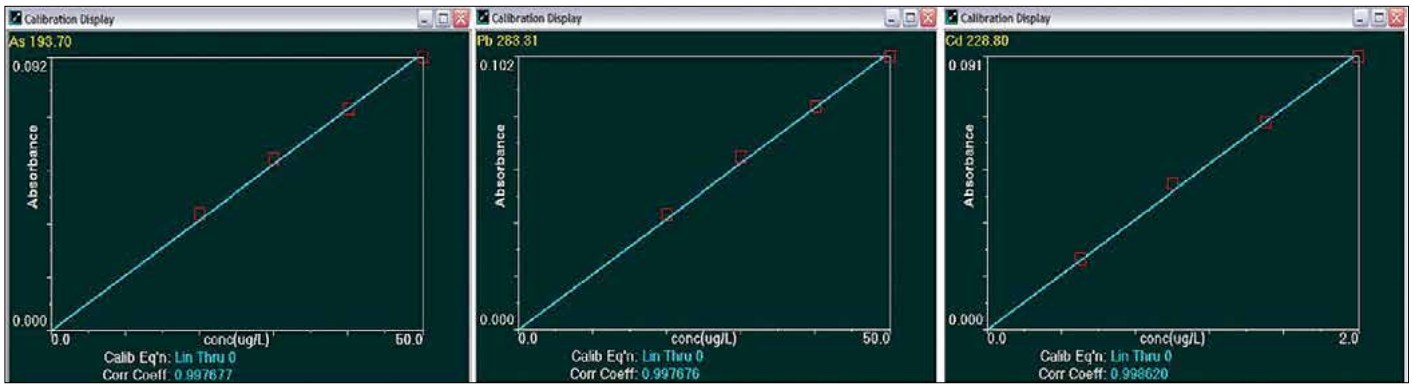

Figure 2. Direct calibration curves for the determination of As, Pb, and Cd in edible oils.

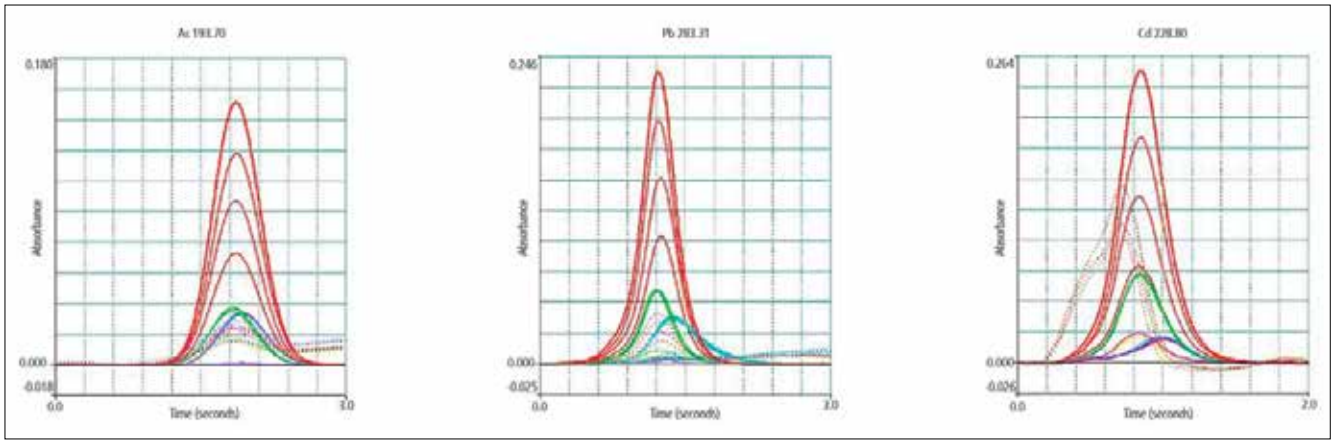

Figure 3. Peak overlays for standards (red), QC checks (green), and samples (various colors) run on the PinAAcle 900T for the analysis of As, Pb, and Cd in edible oils by direct calibration. Solid lines are the analytical signal after background correction (AA-BG); dotted lines are the background signal (BG).

***Table 3.* Result for direct edible oils metals analysis using GFAAS.**

Analyte	As	Pb	Cd
Palm Oil (µg/L)	< MDL	< MDL	< MDL
Sesame Oil (µg/L)	< MDL	< MDL	< MDL
Sunflower Oil (µg/L)	< MDL	< MDL	< MDL
Soybean Oil (µg/L)	4.28	< MDL	< MDL
Rice Bran Oil (µg/L)	< MDL	< MDL	< MDL

Method detection limits (MDLs) were calculated based on 3 times the standard deviation of seven (for Cd and As) or five (for Pb) replicates of the IPA blank. The result was then multiplied by 20, in regards to the 20x sample dilution, to estimate the MDL in standard/sample units. Table 4 shows the resulting MDLs of the PinAAcle 900T spectrometer in analyzing difficult oil matrices at lower concentrations.

***Table 4.* Method detection limits (MDLs) for the analysis of edible oils using the PinAAcle 900T.**

Analyte	As	Pb	Cd
MDL (µg/L)	3.4	3.0	0.42

The goal of this method was to develop a simple and direct method for the quantitative analysis of various toxic metals in edible oils using GFAAS without any sample pretreatment. The validity of the developed method has been verified by incorporating various QC checks and spike recovery checks throughout the analysis. As shown in Table 5, the QC samples showed good recoveries between 98-110%, well within acceptable limits. In addition, individual samples of oil were spiked for either As, Pb, or Cd in concentrations of 10 µg/L, 10 µg/L and 0.5 µg/L respectively. The recoveries for the individual spiked oils were between 93-112% (Table 5) meeting the guidelines of ±15%.

***Table 5.* Recoveries of QC checks and spiked samples for the direct analysis of edible oils using GFAAS.**

	% Recovery		
Analyte	As	Pb	Cd
QC1	104	110	107
QC2	98.4	110	109
QC3	104	109	109
Spike Recovery – Palm Oil	93.9	106	109
Spike Recovery – Sesame Oil	94.8	93.2	112
Spike Recovery – Sunflower Oil	98.8	93.5	108

Conclusions

A direct injection method for the quantitative analysis of toxic elements in edible oil samples was developed. With the THGA tube design, accuracy and sample throughput are improved by reducing the need of time-consuming sample pretreatment. The unique optical system, solid-state detector (which is highly efficient at low wavelengths), THGA, STPF technique, and longitudinal Zeeman background correction, all contribute to the ability of the PinAAcle 900T spectrometer to provide highly accurate, fast and reproducible results with difficult matrices such as edible oils. The PinAAcle 900Z (Longitudinal Zeeman Furnace only) spectrometer can also be used for this application.

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APPLICATION NOTE

UV/Vis Spectroscopy

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Measurement of Quality of Crude Palm Oils used in Margarine Production by UV/Visible Spectroscopy

Crude Palm Oil (CPO) is a raw material used in the production of margarine and other vegetable oil based food products. CPO is traded and there are quality specifications based on free fatty acids (FFAs) as well as moisture and impurities.[1,2,3]

Margarine manufacturers also want to assess the CPO's 'fitness for refining' which is measured by the Deterioration of Bleachability Index (DOBI). A DOBI index of less than 1.8 indicates a poor quality oil; a DOBI index > 3 indicates a high quality oil

The DOBI index is defined as the absorbance ratio $A_{446\ nm}$ / $A_{269\ nm}$ of around 0.04 g oil dissolved in 25 mL of hexane or 2,2,4Trimethylpentane (iso-octane).

Rather than simply measuring the DOBI at fixed wavelengths, there are advantages in measuring the spectrum between 220 and 500 nm as it means that it is also possible to calculate the carotene content by measuring the CPOs primary and secondary oxidation products. In addition, any adulterants added to enhance the DOBI can be detected by examining the spectrum in more detail.

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The primary and secondary oxidation products can be measured at 233 nm and 270 nm, respectively. This is converted into an E1% value by:

$$E_{1\%233\ nm} = \frac{25(A_{233\ nm} - S_{233\ nm})}{100p}$$

Where

$A_{233\ nm}$ = Absorbance of oil

$S_{233\ nm}$ = Absorbance of solvent (normally subtracted automatically by the instrument)

p = weight of palm oil

This value (and the similar value at 270 nm) are then further corrected by also recording the $E_{1\%446\ nm}$ and performing the following correction:

$$E_{1\%233\ nm\ (corrected)} = E_{1\%233\ nm} - 0.06E_{1\%446\ nm}$$

and

$$E_{1\%270\ nm\ (corrected)} = E_{1\%270\ nm} - 0.18E_{1\%446\ nm}$$

Finally, the concentration of carotene in oil (in ppm) can be determined according to the procedure of Cocks and van Rede[4]:

$$\text{Carotene} = 383 - E_{1\%446\ nm}$$

A UV WinLab™ method has been developed to perform these calculations as well as making a pass/fail analysis and is available from PerkinElmer. An example of the software user interface is given in Figure 1.

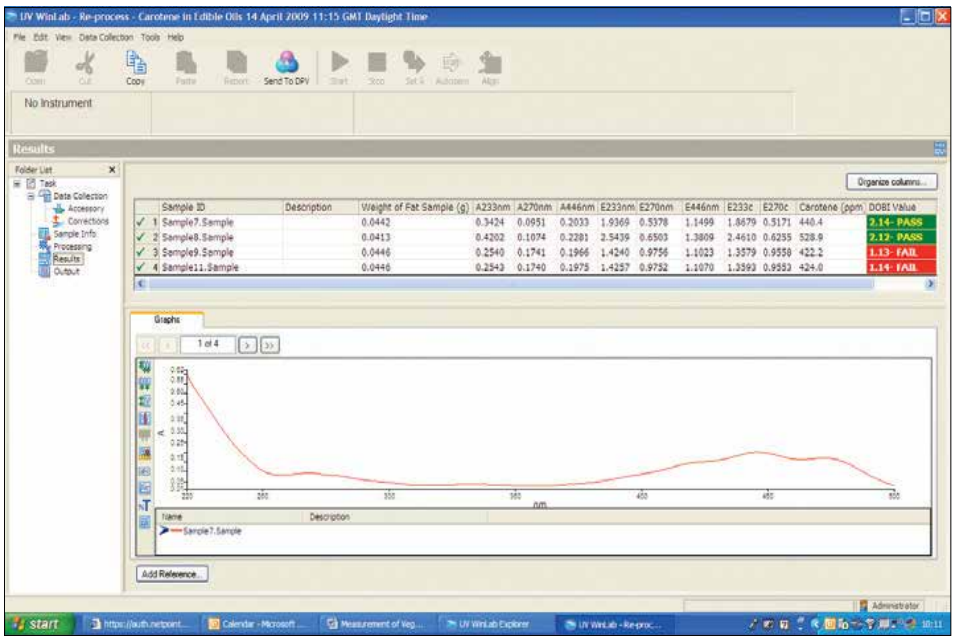

Figure 1. DOBI Analysis using UV WinLab v6 Software.

The method also produces a high quality final report as shown in Figure 2.

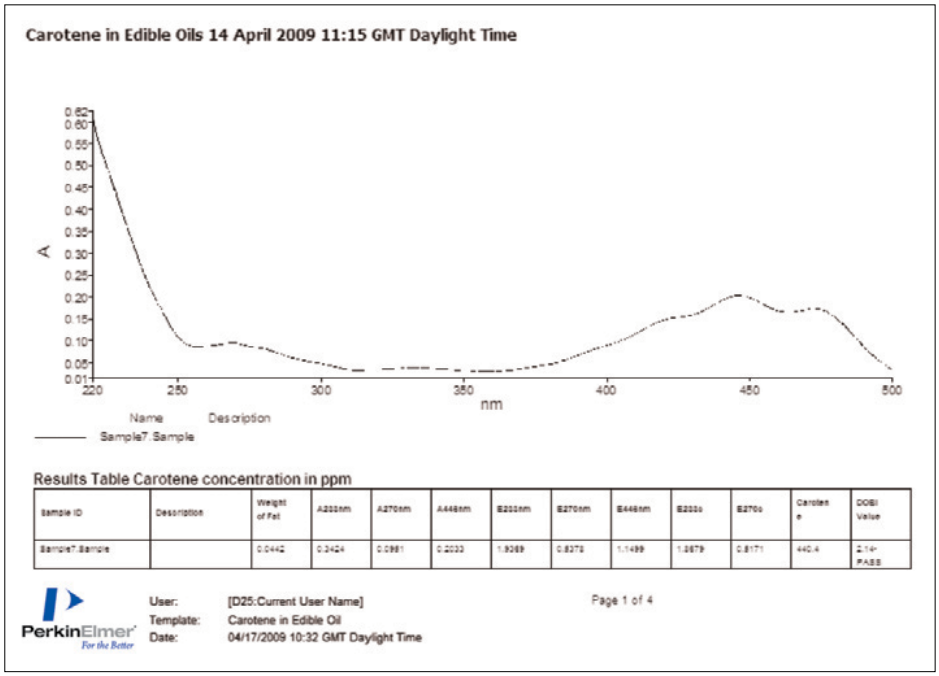

Figure 2. Carotene Concentration and DOBI Index UV WinLab Report.

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