FOOD & BEVERAGE SCIENCE

Application eBook

JASCO

CONTENTS

Classification of Olive Oil (Fluorescence)

In many cases, analyzing the components of food is essential—not only for detecting fraudulent products, but also for verifying the source, species identity, ingredient quality, and even the geographic origin of foodstuffs. Given the often complex composition of food, extracting detailed chemical information typically requires advanced techniques such as chromatography and mass spectrometry. While powerful, these methods are also costly, labor-intensive, and require extensive sample preparation and high technical expertise.

As a faster and more accessible alternative, fluorescence spectroscopy has gained interest due to its minimal sample pretreatment and rapid analysis capabilities. In particular, Excitation-Emission Matrices (EEMs) have become a promising tool for studying food components containing fluorophores. By analyzing the unique fluorescence "fingerprints" in EEMs, researchers can estimate specific components and perform quantitative analysis of complex food mixtures.

To extract meaningful data from these matrices, and decompose and classify the overlapping fluorescence signals, we use parallel factor analysis (PARAFAC), which is a type of multivariate analysis.

In this example, we present results from the fluorescence measurement of several commercial olive oil samples,six brands of extra virgin olive oil and two brands of pure olive oil. The data were analyzed using PARAFAC to successfully classify the samples based on their unique spectral profiles.

Identification of the Components in Olive Oil

Excitation Emission matrices after spectral correction, and Raman rejection of the solvent, are shown in Figure 1 (on the following page). Peak positions in Figure 1, and those found in the literature* were used to estimate the component composition in the samples and are described as the following: oxidation product (Excitation: 300 to 400 nm, Emission: 320 to 500 nm) and chlorophyll (Excitation: 300 to 700 nm, Emission: 650 to 800 nm).

*Kongbonga YGM, Ghalila H, Onana MB, Majdi Y, Lakhdar ZB, Mezlini H and Ghalila SS, *Food and Nutrition Sciences*, **2**, 692-699, (2011).

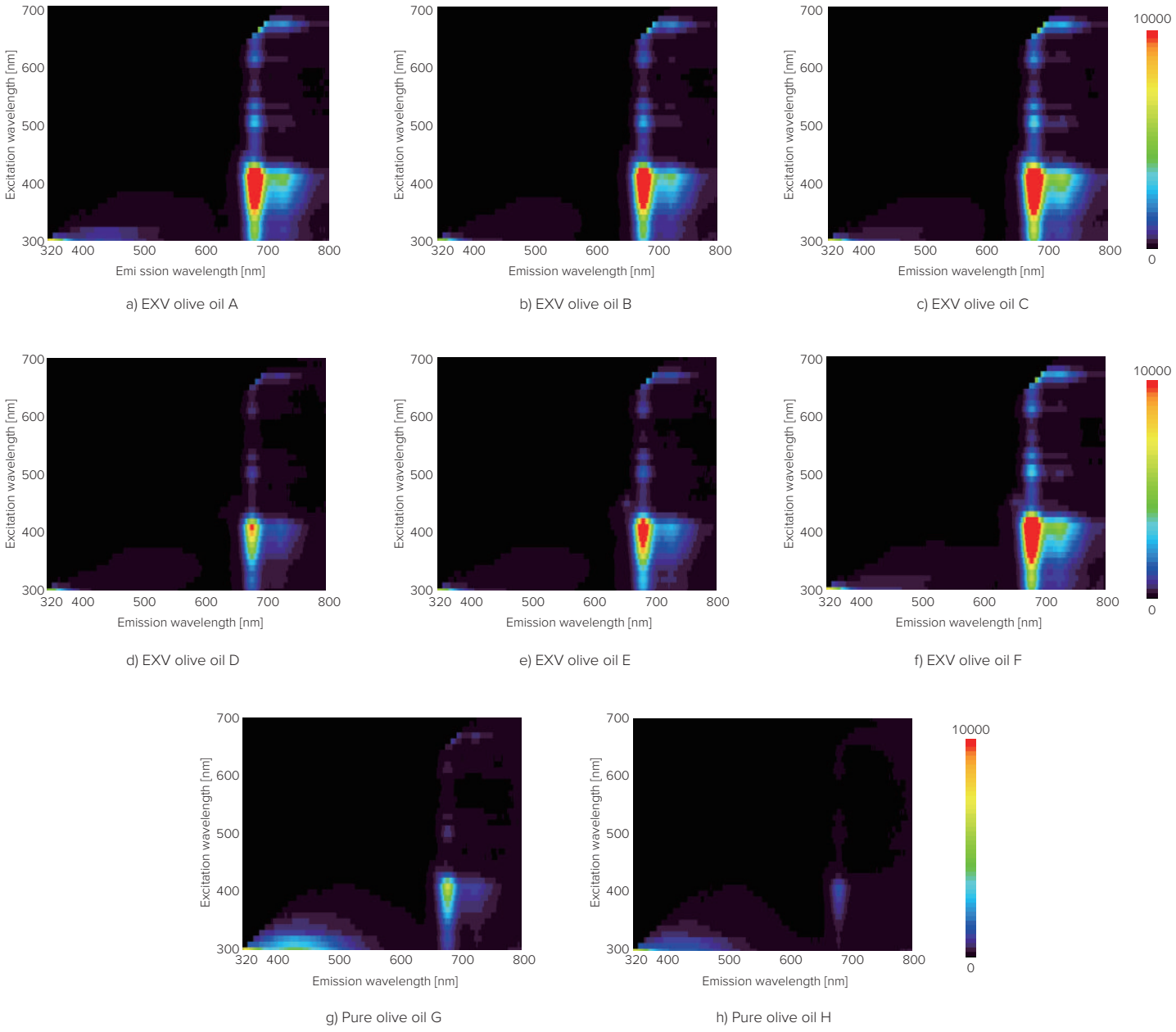


Figure 1. EEM of olive oil samples

PARAFAC was performed on the Excitation Emission matrices, with the expected number of component spectra set to 2. Figure 2 shows the component EEMs calculated using PARAFAC. The first component EEM is chlorophyll and the second is an oxidation product.

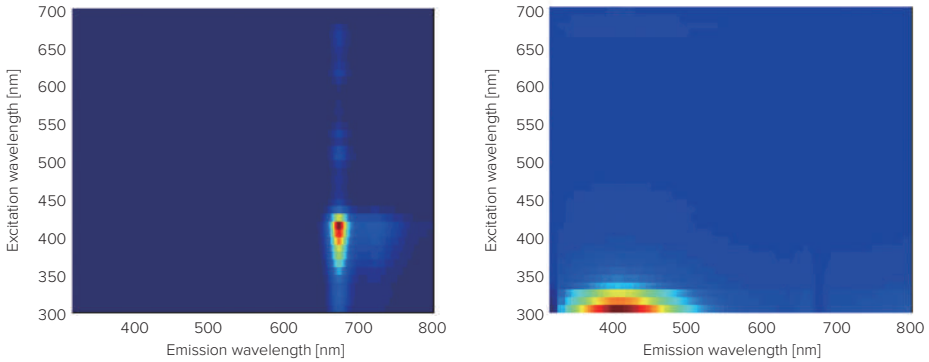


Figure 2. First (left) and second (right) component EEM calculated by PARAFAC.

Classification of Olive Oil

Figure 3 presents the score plot of the first component (chlorophyll) and the second component (oxidation product) extracted from PARAFAC analysis. In the extra virgin olive oil samples, the first component scores high while the second remains low, indicating a high chlorophyll content and low oxidation levels. In contrast, pure olive oil samples show the opposite trend—low scores for chlorophyll and higher scores for oxidation products.

These differences reflect the production methods used. Extra virgin olive oil is obtained by cold-pressing and filtering olives without chemical processing, maintaining a low acidity level (<0.8%) and higher chlorophyll content. Pure olive oil, on the other hand, is a blend of virgin and refined oils, with acidity typically below 1%. During refining, chlorophyll is partially removed, resulting in lower chlorophyll levels and higher oxidation.

PARAFAC analysis of EEMs proves to be a powerful tool for discriminating between food products and identifying their key chemical components. This technique is valuable for ingredient identification, quality control monitoring, and detecting food fraud.

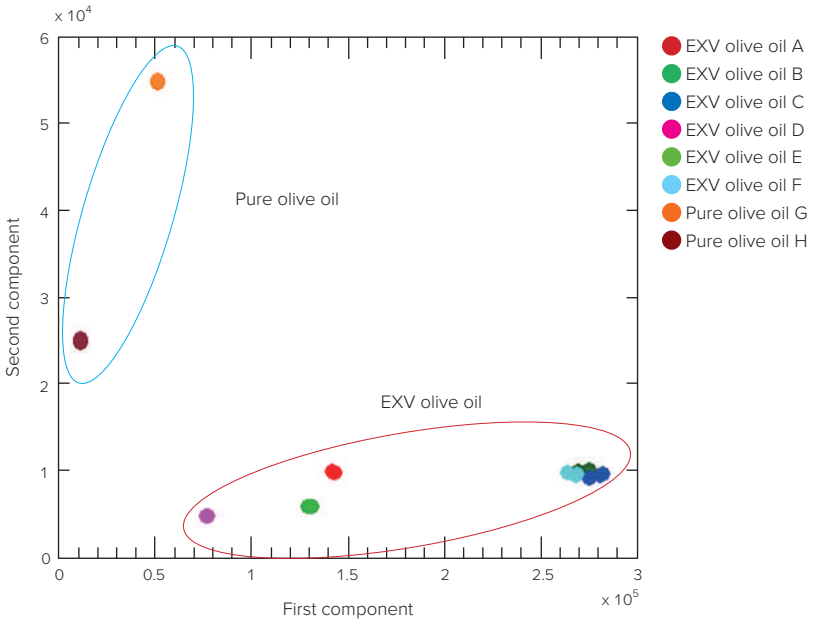


Figure 3. Score plot of the first and second components analyzed using PARAFAC

Quantitative Analysis of Free Fatty Acids in Vegetable Oil using ADAM Derivatization (UHPLC)

High-Performance Liquid Chromatography (HPLC) is a widely used technique for the quantitative analysis of fatty acids in oils and lipids. These components are commonly measured using Ultraviolet (UV) absorbance, Refractive Index (RI), or Evaporative Light Scattering Detection (ELSD). When target analytes are present at very low concentrations, derivatization is often employed to enhance sensitivity and improve detection limits. Among the available derivatization reagents, 9-Anthryldiazomethane (ADAM) is one of the most effective and commonly used for fatty acid analysis. ADAM readily reacts with fatty acids at room temperature, forming highly stable fluorescent derivatives that enable highly sensitive and selective detection by fluorescence.

This section shows the separation and quantification of free fatty acids in vegetable oil using Ultra High-Performance Liquid Chromatography (UHPLC) with ADAM derivatization.

Pre-Column Derivatization with ADAM

Figure 1 shows the ADAM derivatization procedure, and Figure 2 shows the reaction mechanism of the ADAM reagent with fatty acids. In this section, ADAM derivatization is performed prior to HPLC injection (pre-column derivatization). The ADAM reagent reacts with the carboxylic acid group of the fatty acid, forming a stable and fluorescent ADAM-derivatized fatty acid. This derivative enhances detection sensitivity and enables accurate quantification, even at trace levels.

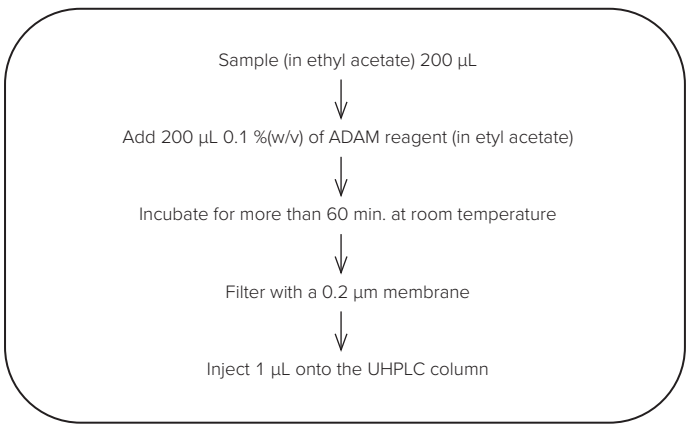


Figure 1. ADAM derivatization procedure

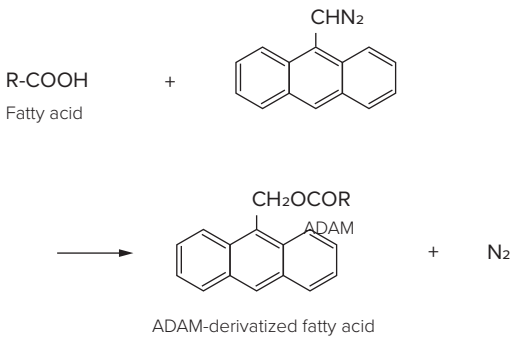
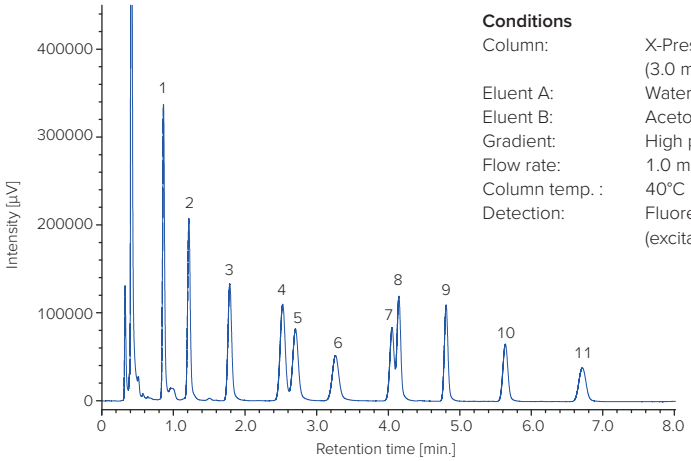


Figure 2. Reaction mechanism of the ADAM reagent with fatty acids

1. Mix 200 µL of sample with 200 µL of 0.1 %(w/v) of ADAM reagent (both in ethyl acetate), 2. Incubate at room temperature for at least 60 minutes to allow complete derivatization, 3. Filter the reaction mixture through a 0.2 µm membrane filter to remove particulates, 4. Inject 1 µL of the derivatized sample onto the UHPLC column for analysis

Analysis of Standard Fatty Acids

Figure 3 shows the chromatogram of a standard mixture of fatty acids derivatized with the ADAM reagent. All eleven fatty acids were separated in under 7 minutes.



Conditions

Column:	X-PressPak V-C18-W (3.0 mmI.D. × 50 mmL, 2.0 µm)
Eluent A:	Water/Methanol (5/95)
Eluent B:	Acetonitrile/THF (90/10)
Gradient:	High pressure gradient elution
Flow rate:	1.0 mL/min.
Column temp. :	40°C
Detection:	Fluorescence detector (excitation: 365 nm, emission: 412 nm)

Figure 3. Chromatogram of standard mixture of fatty acids derivatized with the ADAM reagent

1: Caprylic acid (C8), 2: Capric acid (C10), 3: Lauric acid (C12), 4: Linolenic acid (C18:3), 5: Myristic acid(C14), 6: Linoleic acid (C18:2), 7: Palmitic acid (C16), 8: Oleic acid (C18:1), 9: Stearic acid (C18),10: Arachidic acid (C20), 11: Behenic acid (C22)

Analysis of Fatty Acids in Vegetable Oils

Figure 4 shows the chromatograms of rice oil and coconut oil derivatized with the ADAM reagent. Table 1 shows the quantitative values (in mg/g) of each fatty acid found in the rice and coconut oil.

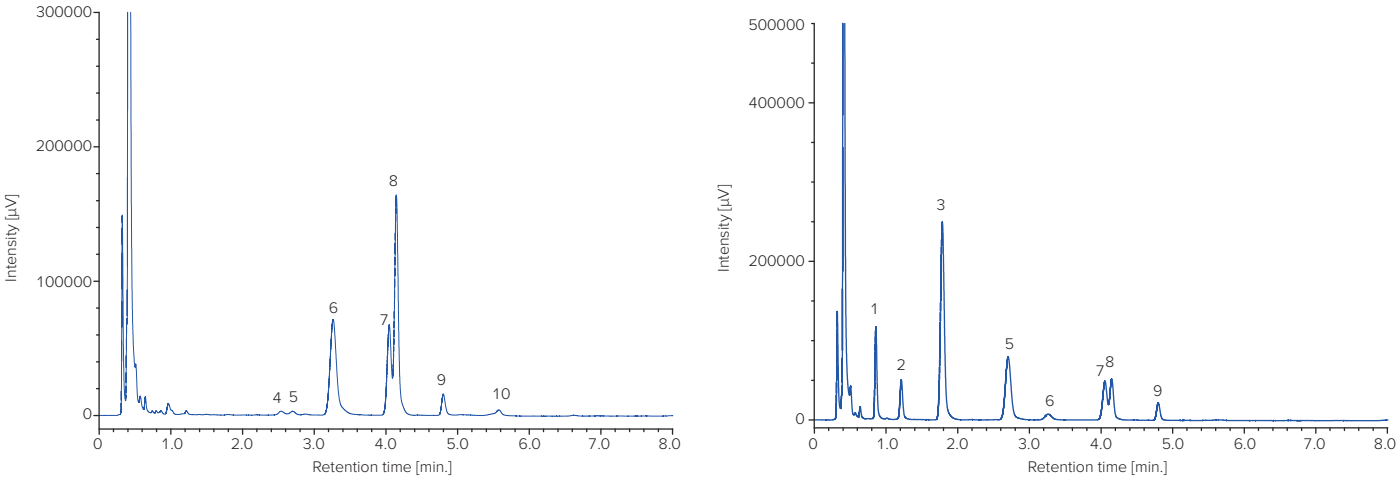


Figure 4. Chromatograms of ADAM-derivatized fatty acids in rice oil (left) and coconut oil (right)

Chromatographic conditions and peak numbers are the same as in Figure 3.

Preparation: 1.0 g of oil was dissolved in ethyl acetate and brought to a final volume of 10 mL. The sample was derivatized with ADAM according to the procedure in Figure 1.

Table 1. Quantitative values (mg/g) of rice oil and coconut oil

Fatty oil	C8	C10	C12	C14	C16	C18	C18:1	C18:2	C18:3	C20	C22
Rice oil	-	-	-	0.010	0.23	0.044	0.41	0.38	0.007	0.020	-
Coconut oil	0.017	0.012	0.85	0.45	0.26	0.089	0.20	0.055	-	-	-

Component Analysis of Dairy Products (FTIR)

Understanding the nature and composition of food ingredients is essential for solving problems in the manufacturing process and maintaining product quality control. Infrared (IR) spectroscopy is a powerful analytical technique that can provide detailed information about food components. However, due to the complex mixture of ingredients typically present in food, the resulting IR spectra can be difficult to interpret directly.

To extract meaningful insights from these complex spectral datasets, chemometric methods are often applied. Two of the most widely used algorithms in this context are Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression.

PCA reduces the dimensionality of spectral data while preserving its most important information. By identifying latent variables—underlying patterns within the data—PCA enables effective classification and identification of sample characteristics.

PLS regression also performs dimensionality reduction, but unlike PCA, it focuses on the relationship between predictor variables (e.g., spectral data) and a response variable (e.g., concentration). By maximizing the covariance between these variables, PLS builds robust predictive models that are less sensitive to noise and more effective than standard multiple regression techniques.

In this article, we present two examples using IR spectroscopy:

- PCA for component classification in a dairy product
- PLS regression for quantitative protein analysis in a food sample

These examples highlight how the combination of IR spectroscopy and chemometric analysis provides powerful tools for food quality assessment and ingredient verification.

Component Analysis of Milk by PCA

IR spectra were collected using an FTIR spectrometer equipped with ATR (Attenuated Total Reflectance) measurement. Figure 1 displays the IR difference spectra between milk and water. Since milk is composed of approximately 90% water, with the remainder consisting primarily of proteins, sugars, and fats, subtracting the water spectrum from the milk spectrum allows the characteristic absorption bands of these other components to be more clearly observed. This approach highlights the functional groups associated with each major ingredient in milk.

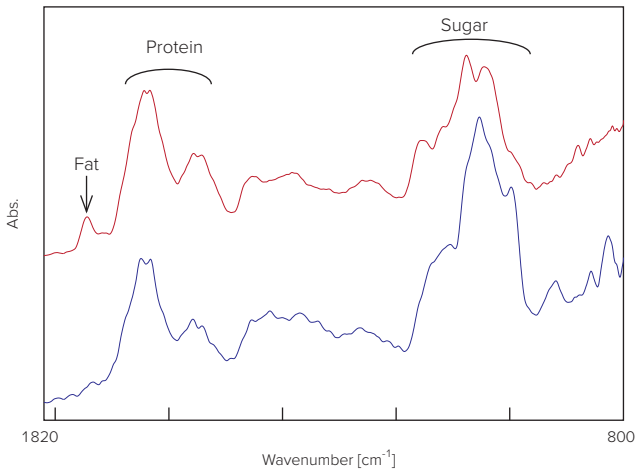


Figure 1. IR difference spectra between milk and water
(red: milk, blue: coffee with milk)

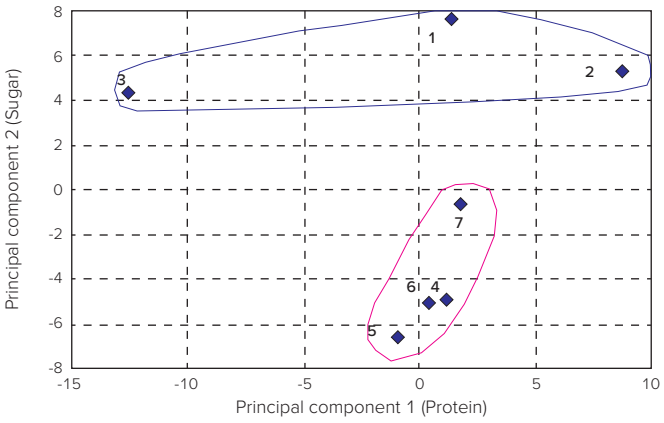


Figure 2. Principal component scores
1. Coffee with milk (A), 2. Coffee with milk (B), 3. Coffee with milk (C), 4. Milk (D), 5. Milk (E), 6. Milk (F), 7. Milk (G)

Next, 7 different commercial milk samples were measured, and classification was made using PCA (Figure 2). Figure 2 indicates that the component ratios of milk samples (Nos. 4 to 7) are almost the same. Regarding the coffee with milk samples (Nos. 1 to 3), it is indicated that the sugar ratios are also similar, but they are largely different in the other component ratios.

Quantitative Analysis of Protein in Food Products

Several food samples including protein (table 1) were measured using IR spectroscopy. Figure 3 shows the near-IR spectrum of each. Although these spectra are similar, there are slight differences between them. Next, a PLS calibration model was created using the measured data and the protein concentration value obtained from the labeling on each food sample (table 1). Figure 4 shows the correlation between the spectrum and protein concentration. Its correlation coefficient was 0.994, suggesting a good calibration model.

Table 1. Food samples and their protein concentration (on label)

Food Sample	Protein Concentration
Gouda cheese	18.0 %
Natural cheese	28.0 %
Worcester sauce	0.9 %
Yogurt	4.2 %
Soybean milk yogurt	4.0 %
Miso (salted bean-paste)	10.0 %

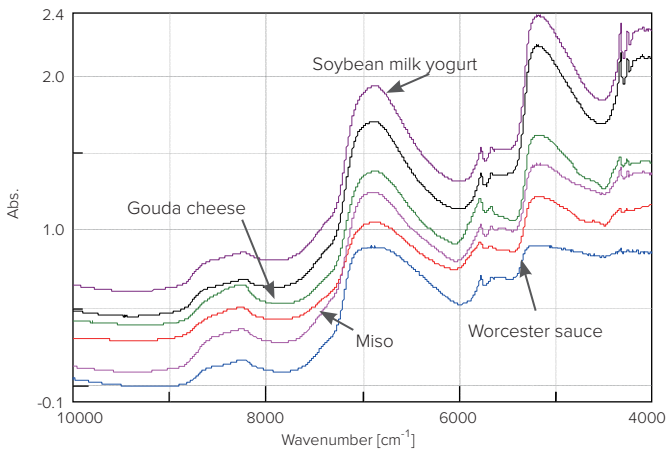


Figure 3. Near-IR spectra of food samples

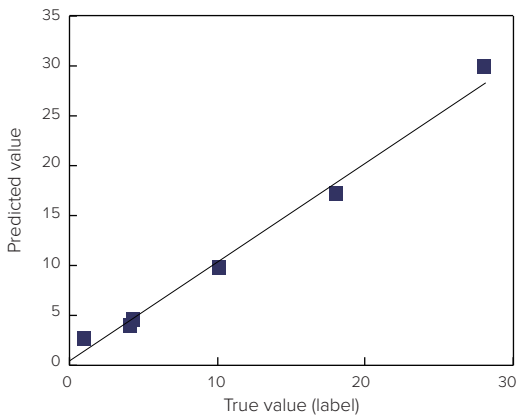


Figure 4. PLS calibration model of protein

Simultaneous Quantitative Analysis of Multi-Components in Food Products

Near-Infrared (NIR) spectroscopy is widely used for the quantitative analysis of components in food products, offering a key advantage: non-destructive measurement with minimal to no sample preparation. This technique is highly versatile, accommodating various physical states of samples—whether liquid, paste, solid, or particulate—by simply placing the sample on a measurement plate or into a sample cell.

Additionally, by applying multivariate analysis, NIR spectroscopy enables the simultaneous quantification of multiple components from a single spectrum. This eliminates the need to separate or deconvolute overlapping spectral data, allowing for rapid and comprehensive analysis.

This application note highlights the use of NIR spectroscopy for the simultaneous quantitative analysis of multiple components in a range of commercial food products, demonstrating both the efficiency and effectiveness of this approach.

Simultaneous Quantitative Analysis of Multi-Components in Condensed Soup

Fifteen different condensed soup samples with known component concentrations were prepared, and diffuse transmission measurements were conducted using a UV-Visible-NIR spectrophotometer equipped with an integrating sphere. Figure 1 shows the spectra of all samples. While the overall spectral patterns appear similar, subtle differences can be observed between them.

Using the measured data, a partial least squares (PLS) regression model was developed to quantify salt (NaCl) concentration (Figure 2). Despite the fact that salt itself does not exhibit distinct absorption features in the near-IR region, it can still be quantified by analyzing indirect spectral effects—specifically, the shifts in water absorption peaks caused by varying salt concentrations. The resulting calibration model demonstrated a high correlation coefficient, indicating strong predictive performance.

In addition to salt, calibration models for water and protein content were also developed using the same PLS method. Table 1 summarizes the correlation coefficients for each component. The results confirm that this approach enables the simultaneous quantification of multiple components in complex food matrices with high accuracy.

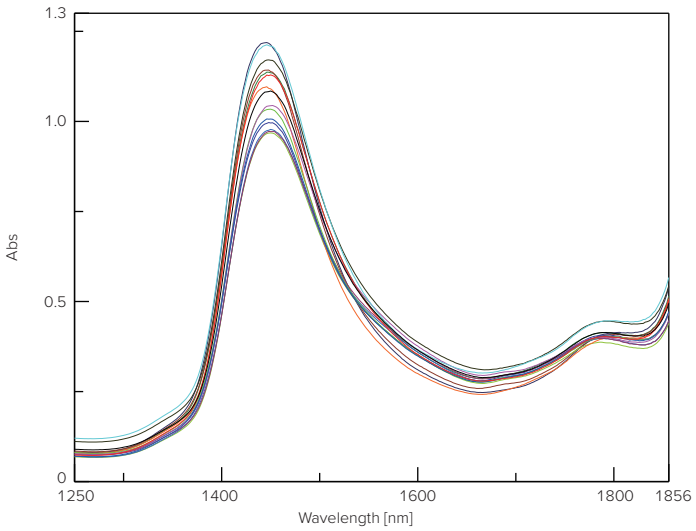


Figure 1. Spectra of each condensed soup

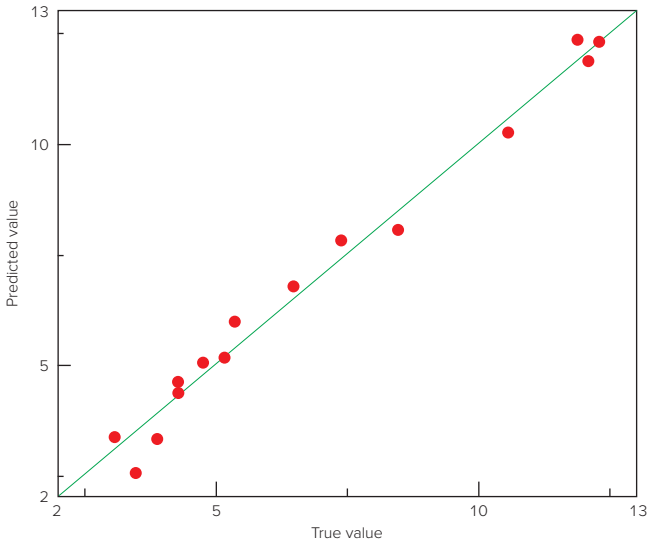


Figure 2. PLS calibration model of salt

Table 1. Calibration model of each component and their correlation coefficient

Component	Number of samples	Range (%)	Correlation coefficient	Error of prediction
Water	15	55.7 to 77.4	0.961	2.04
Protein	15	1.0 to 4.7	0.955	0.470
Salt	15	3.1 to 12.3	0.992	0.415

Simultaneous Quantitative Analysis of Multi-Component in Soybeans

Soybean samples were analyzed non-destructively using a Fourier Transform Near-Infrared (FT-NIR) spectrometer. Figure 3 displays the NIR spectra of each sample. While the overall spectral profiles appear similar, subtle differences can be observed, reflecting variations in composition among the samples.

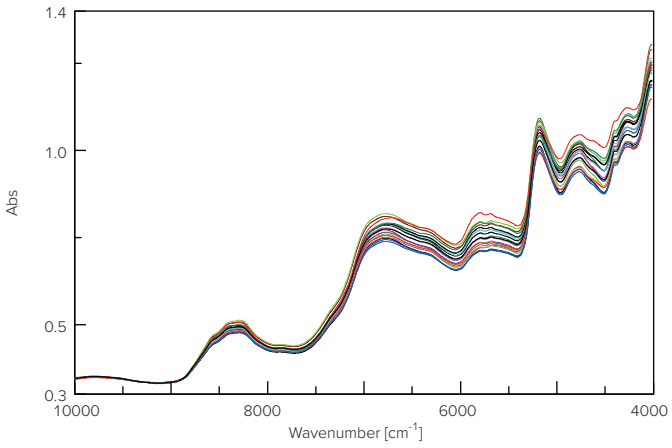


Figure 3. Near-IR spectra of each soybean

Next, PLS calibration models were developed for each component using the measured near-IR spectra. The reference values for each component—protein, water, carbohydrate, and fat—were obtained through standard wet chemistry methods and used as the ground truth in building the calibration models.

Figure 4 shows the resulting PLS models, which demonstrate strong correlation between the predicted values and the reference values for all components. These results indicate that the method enables accurate simultaneous quantification of multiple components from a single near-IR measurement, highlighting its effectiveness for rapid, non-destructive food analysis.

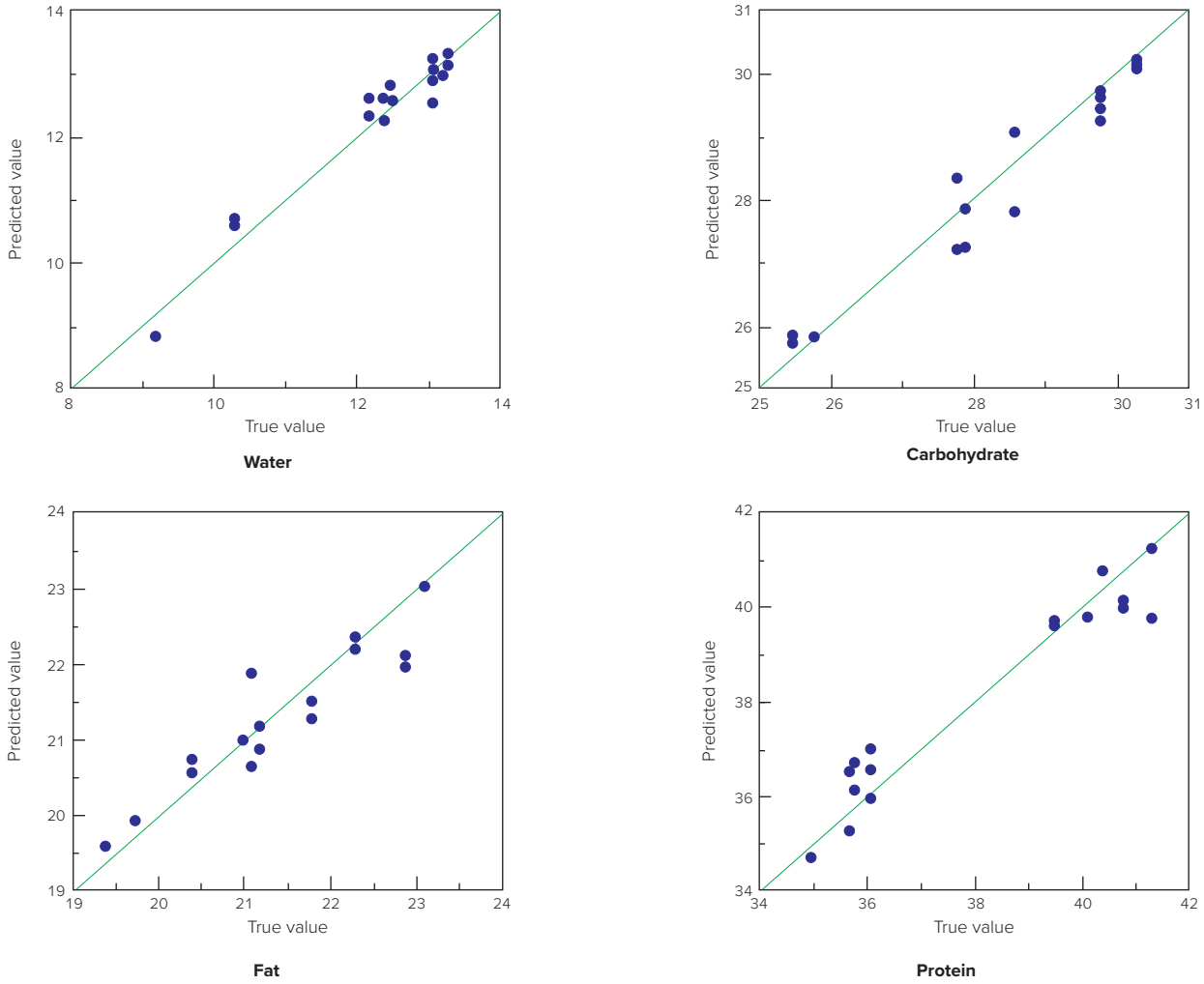


Figure 4. PLS calibration models

An FT-NIR system has the following advantage compared to other spectroscopy systems and can be used for quantifying the multi-components in food product simultaneously.

- Excellent wavenumber accuracy
- Bright and high throughput
- Continuous measurement and monitoring available
- Simultaneous measurement in the entire region

Quantitative Amino Acid Analysis in Functional Beverages using OPA Derivatization (HPLC)

High-Performance Liquid Chromatography (HPLC) is one of the most effective and widely used techniques for amino acid analysis as a simple and rapid solution. Since most amino acids lack natural chromophores or fluorophores, they do not exhibit significant absorption or fluorescence in the UV-Visible range. These amino acids must first be derivatized using a suitable reagent to enable their detection with high sensitivity.

A commonly used derivatizing reagent is o-phthalaldehyde (OPA), which selectively reacts with primary amino acids to form highly fluorescent derivatives. This derivatization reaction attaches a fluorophore to the amino acid, enabling highly sensitive and selective quantification by either UV-Visible absorbance or fluorescence detection. This section shows the HPLC analysis of OPA-derivatized amino acids in functional beverages using both detection methods.

Derivatization with OPA

Figure 1 shows the reaction mechanism between OPA and primary amino acids. The derivatization proceeds rapidly at room temperature, forming highly fluorescent derivatives; however, the stability of these derivatives can vary depending on the specific amino acid, and in some cases, degradation may occur shortly after formation. To ensure accurate and reproducible results, consistent sample handling and timing are essential.

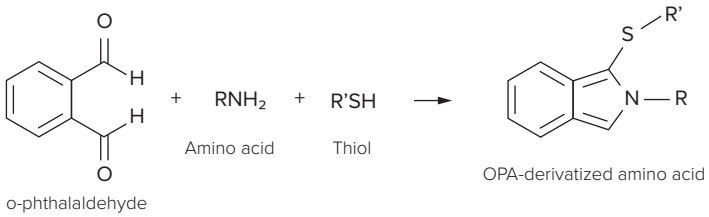
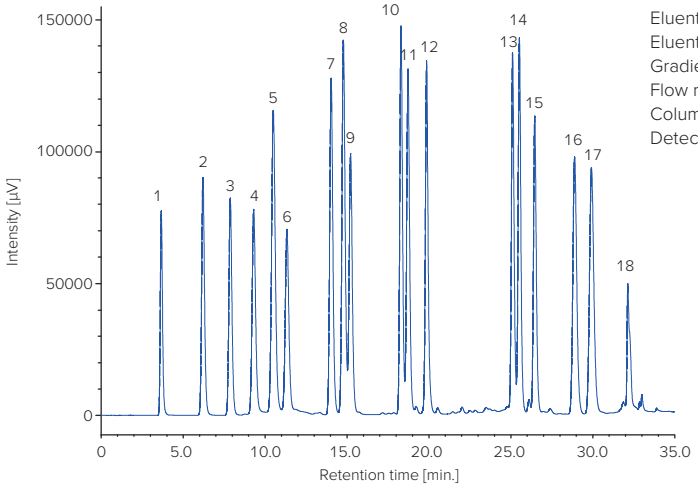


Figure 1. Reaction mechanism of the OPA reagent with an amino acid

OPA-derivatized amino acids exhibit strong fluorescence with detection limits in the femtomole (fmol) range when using an excitation wavelength of 340 – 350 nm and an emission wavelength of 450 – 460 nm. In this section, an autosampler was used to automate the OPA derivatization prior to injection (pre-column derivatization), enabling high reproducibility and minimizing sample handling. The derivatized samples were analyzed by HPLC using both fluorescence and UV absorbance detection.

Amino Acid Analysis by Fluorescence Detection

OPA-derivatized amino acids were analyzed by fluorescence detection using an excitation wavelength of 245 nm and an emission wavelength of 455 nm. Figure 2 shows the chromatogram of a standard mixture of amino acids with excellent resolution and peak symmetry. Figure 3 shows the chromatogram of a functional beverage, demonstrating the method's applicability to real-world samples and its high sensitivity for complex matrices.



Conditions

Column:	CrestPak C18S (4.6 mmI.D. × 150 mmL, 5 µm)
Eluent A:	Sodium acetate buffer/methanol/THF (89/10/1)
Eluent B:	Methanol/THF (90/10)
Gradient:	High pressure gradient elution
Flow rate:	1.0 mL/min.
Column temp.:	20°C
Detection:	Fluorescence detector (excitation: 345 nm, emission: 455 nm)

Figure 2. Chromatogram of a standard mixture of amino acids

1: Aspartic acid, 2: Glutamic acid, 3: Asparagine, 4: Histidine, 5: Serine, 6: Glutamine, 7: Arginine, 8: Glycine, 9: Threonine, 10: Taurine, 11: Alanine, 12: Tyrosine, 13: Methionine, 14: Valine, 15: Phenylalanine, 16: Isoleucine, 17: Leucine, 18: Lysine

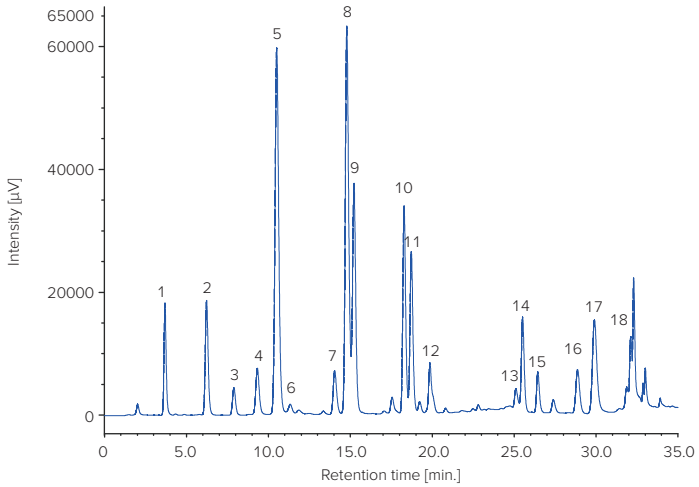


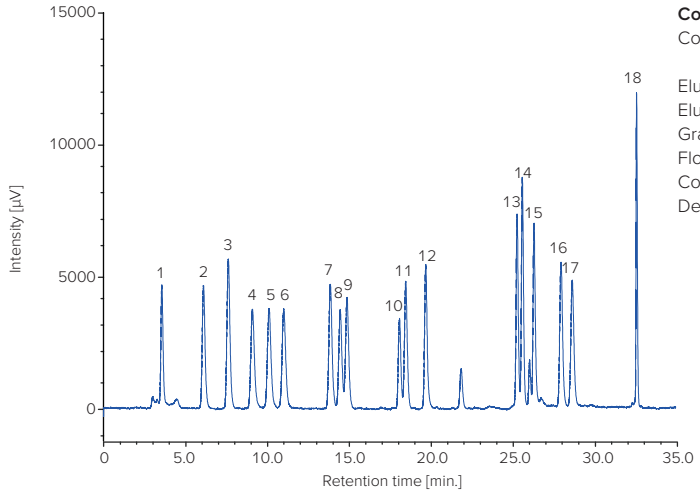
Figure 3. Chromatogram of a functional beverage

Chromatographic conditions and peak numbers are the same as in Figure 2.

Preparation: The functional beverage was diluted 100 times with 0.01 M HCl, then filtered through a 0.2 µm membrane filter. A sample volume of 10 µL was injected.

Amino Acid Analysis by UV Detection

Although fluorescence detection offers superior sensitivity, UV detection provides a practical alternative when a fluorescence detector is unavailable. OPA-derivatized amino acids were analyzed at an absorption wavelength of 338 nm. Figures 4 and 5 show the chromatograms of a standard mixture of amino acids and a functional beverage, respectively, demonstrating good sensitivity and well-resolved peaks. This confirms UV absorbance detection as a viable option for routine amino acid analysis.



Conditions

Column:	CrestPak C18S (4.6 mmI.D. × 150 mmL, 5 µm)
Eluent A:	Sodium acetate buffer/methanol/THF (89/10/1)
Eluent B:	Methanol/THF (90/10)
Gradient:	High pressure gradient elution
Flow rate:	1.0 mL/min.
Column temp.:	20°C
Detection:	UV detector (absorption wavelength: 338 nm)

Figure 4. Chromatogram of a standard mixture of amino acids

1: Aspartic acid, 2: Glutamic acid, 3: Asparagine, 4: Histidine, 5: Serine, 6: Glutamine, 7: Arginine, 8: Glycine, 9: Threonine, 10: Taurine, 11: Alanine, 12: Tyrosine, 13: Methionine, 14: Valine, 15: Phenylalanine, 16: Isoleucine, 17: Leucine, 18: Lysine

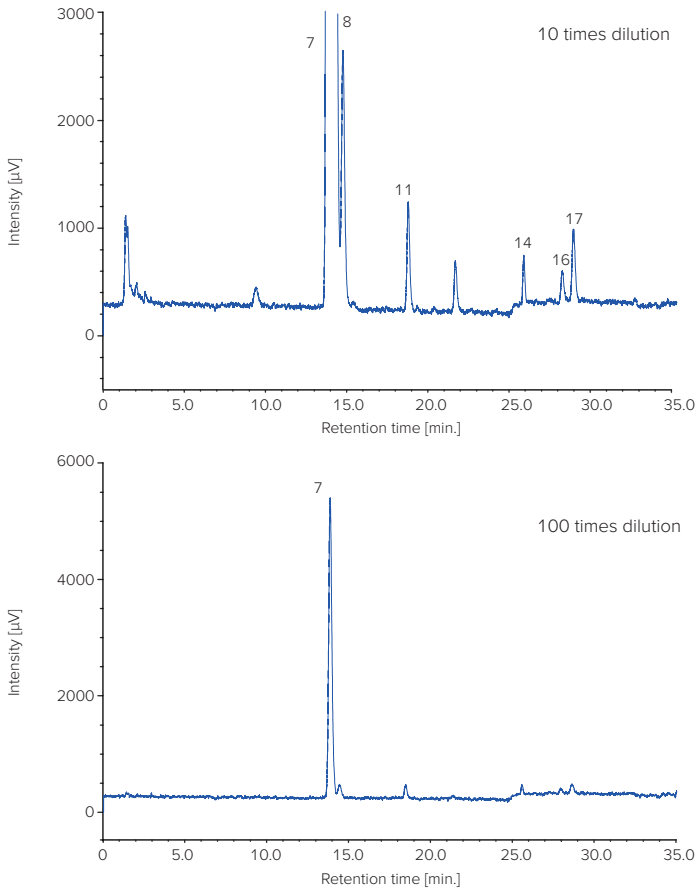


Figure 5. Chromatogram of a functional beverage

Chromatographic conditions and peak numbers are the same as in Figure 4.

Preparation: Functional beverage was diluted 10 times and 100 times with 0.01 M HCl respectively. A sample volume of 5 µL was injected.

Molecular Weight Distribution Analysis of Chondroitin Sulfate C Sodium Salt using Size Exclusion Chromatography (HPLC)

Chondroitin sulfate is a mucopolysaccharide composed of repeating disaccharide units and exits in several structural forms (designated A through E) with molecular weights (MW) typically ranging from 20,000 to 50,000 Da. It is naturally found in the connective tissues of animals, such as cartilage and skin, and commonly used in health foods and supplements due to its physiological functions and health benefits.

This section shows the molecular weight distribution analysis of chondroitin sulfate C sodium salt using High-Performance Liquid Chromatography (HPLC) with Size Exclusion Chromatography (SEC). Separation was performed on a column with a molecular weight exclusion limit of 300,000 Da (calibrated with two pullulan standards), and detection was performed using a Refractive Index (RI) detector. Molecular weight values were calculated using a standard calibration curve derived from the two pullulan standards.

Figure 1 shows the chromatogram of the chondroitin sulfate C sodium salt sample.

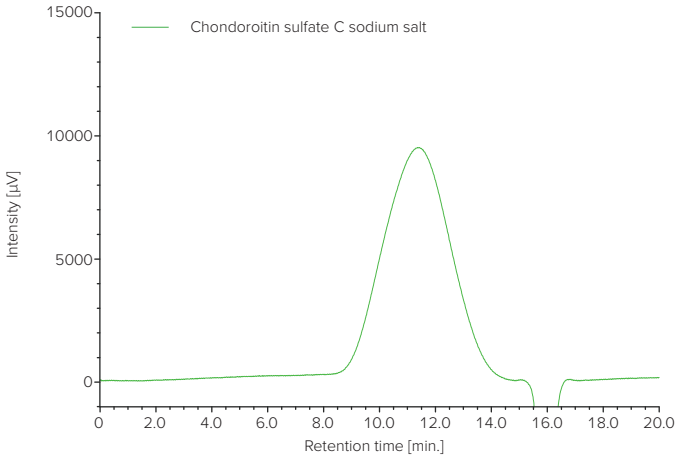


Figure 1. Chromatogram of chondroitin sulfate C sodium salt

Chromatographic conditions are the same as in Figure 2.

Figure 2 shows the chromatograms of two pullulan standard mixtures used to create the molecular weight calibration curve shown in Figure 3. This calibration curve plots retention volume (elution time) against the peak top molecular weight of the pullulan standards.

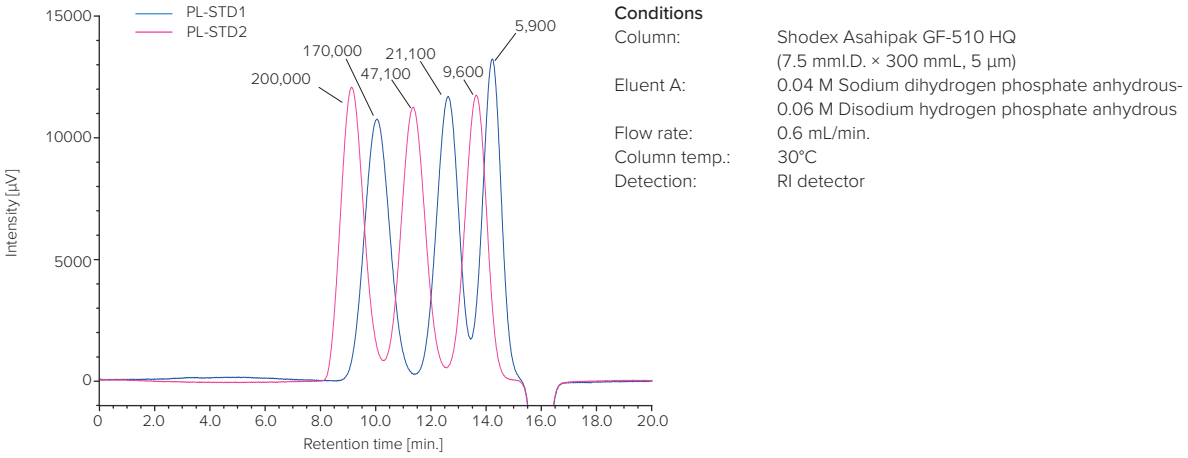


Figure 2. Chromatograms of two standard mixtures of pullulan

(value on the chromatogram is M_p (peak top molecular weight))

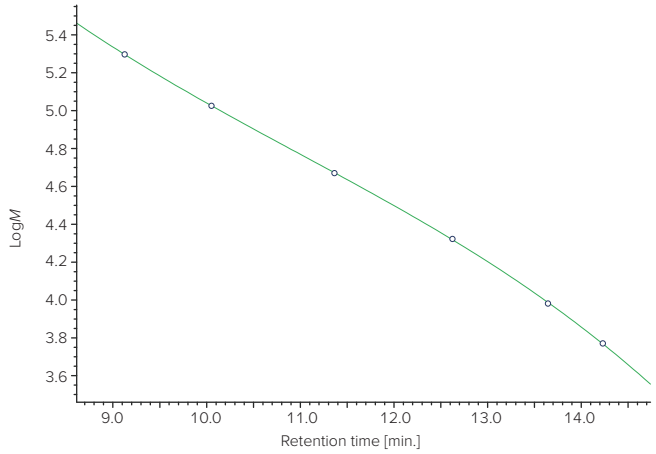


Figure 3. Molecular weight calibration curve created from the two standard mixtures of pullulan

In figure 4, the chromatogram from figure 1 is overlaid with the calibration curve from figure 3, allowing the molecular weights of the sample to be calculated based on the retention time of the chromatogram.

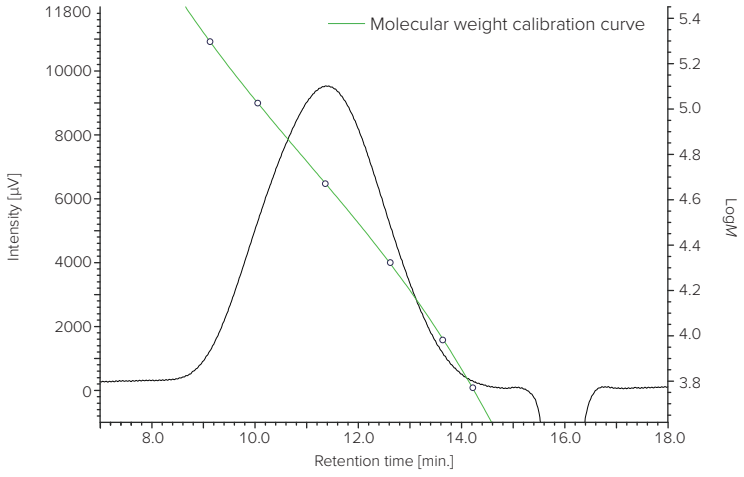


Figure 4. Chromatogram of chondroitin sulfate C sodium salt and molecular weight calibration curve of standard pullulan

(The figure in black represents M_p calculated using the standard mixtures of pullulan)

Figure 5 shows the calculated differential (dMWD) and integral (iMWD) molecular weight distribution curves of the chondroitin sulfate C sodium salt sample based on the overlaid data in Figure 4. Both distributions plot the base-10 logarithm of molecular weight on the x-axis. The iMWD shows the cumulative percentage of the sample composed of molecules below a specific molecular weight, while the dMWD shows the relative abundance of molecular weights in the sample.

When normalized, the differential molecular weight distribution curve facilitates the comparison of a chromatogram and a molecular weight distribution curve that were obtained using different columns or experimental conditions.

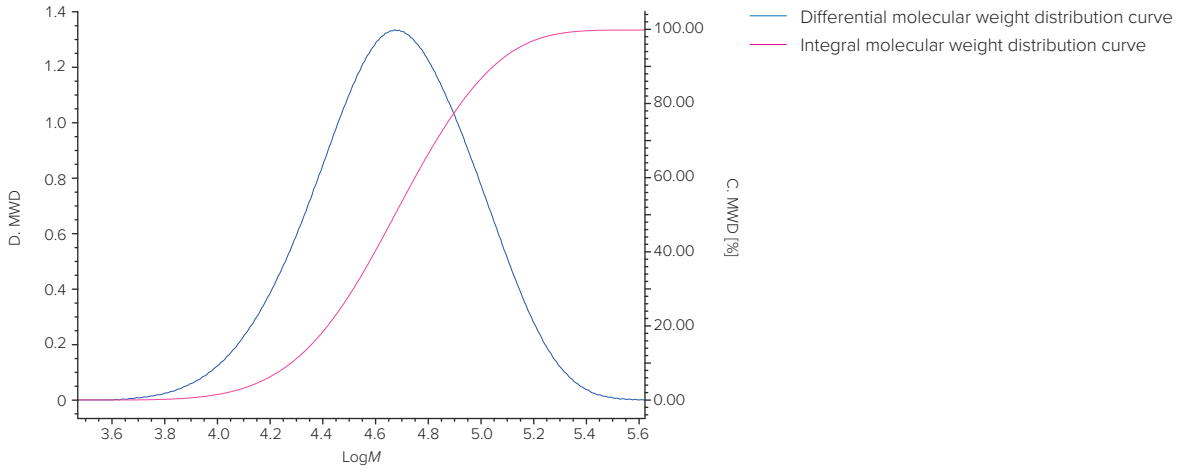


Figure 5. Molecular weight distribution curves of chondroitin sulfate C sodium salt

Table 1 shows the calculated molecular weight averages of a chondroitin sulfate C sodium salt sample based on the pullulan molecular weight calibration curve. The molecular weight averages of polymer materials obtained by SEC includes the number-average (Mn), weight average (Mw), z-average (Mz), and viscosity-average (Mv) molecular weights. The distribution of these molecular weight averages generally follows a relationship, such that Mn ≤ Mv ≤ Mw ≤ Mz. In cases where all the molecular weight averages are equal (Mn = Mv = Mw = Mz), the sample is considered monodisperse, indicating a uniform molecular weight distribution with no measurable polydispersity.

Table 1. Molecular weight of chondroitin sulfate C sodium salt

M_p	M_n	M_w	M_z	M_v	M_w/M_n	M_z/M_w
46812	36668	57914	85947	57914	1.58	1.48

Quantitative Analysis of Sugars (HPLC)

High-Performance Liquid Chromatography (HPLC) is a widely used technique for the quantitative analysis and quality control of sugars in food products. Depending on the types of sugars being analyzed, several chromatographic modes can be employed, including Hydrophilic Interaction Liquid Chromatography (HILIC), Size Exclusion Chromatography (SEC), Ion-Exchange Chromatography (IEC), and Ligand-Exchange Chromatography (LEC).

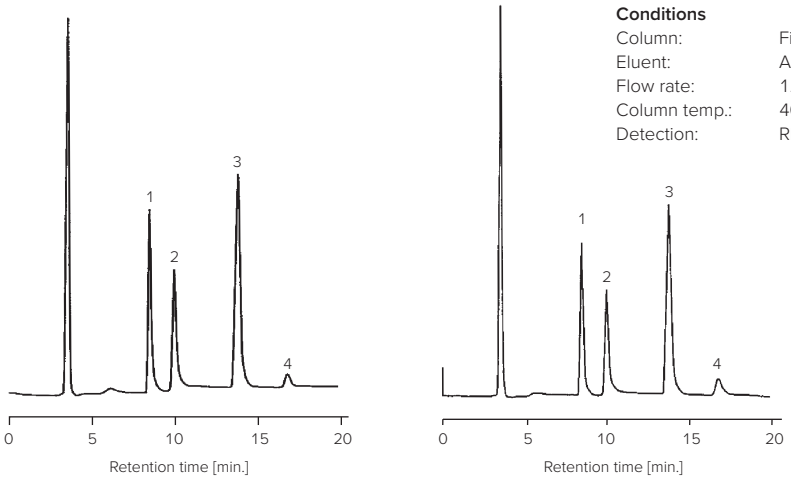
Since sugars lack natural chromophores, they do not absorb light in the UV-Visible region. As a result, Refractive Index (RI) detectors and Evaporative Light Scattering Detectors (ELSD) are commonly used for detection. Alternatively, sugars can be chemically derivatized to introduce a chromophore or fluorophore, enabling more sensitive detection by UV or fluorescence detectors. This section shows two different HPLC-based approaches for sugar analysis: direct detection using a RI detector and post-column derivatization using a fluorescence detector.

Direct Detection of Sugars using a RI Detector

RI detectors measure the difference in the refractive index between the pure mobile phase and the mobile phase with eluting sample components, making it an ideal detection method for non-UV absorbing compounds, such are sugars. HILIC columns are commonly used for sugar separation due to their affinity for polar compounds.

The JASCO RI detector has excellent baseline stability, enabled by precise flow cell temperature control that minimizes the impact of environmental fluctuations, and is compatible with a wide range of applications from the analytical to semi-micro and preparative scale.

Figure 1 shows the chromatograms of strawberry jam and orange marmalade samples. Table 1 shows the quantitative values of four sugars – fructose and glucose (monosaccharides) as well as sucrose and maltose (disaccharides) – found in the strawberry jam and orange marmalade samples. All four sugars were well resolved and accurately quantified.



Conditions

Column:	Finepak SIL NH_2
Eluent:	Acetonitrile/Water (75/25)
Flow rate:	1.0 mL/min.
Column temp.:	40°C
Detection:	RI detector

Figure 1. Chromatograms of strawberry jam (left) and orange marmalade (right) (1: Fructose, 2: Glucose, 3: Sucrose, 4: Maltose)

Preparation: 1.0 g of sample was weighed, and 5 mL of water was added. Then, filtered through a 0.45 µm membrane filter. A sample volume of 5 µL was injected.

Table 1. Quantitative values of sugars in strawberry jam and orange marmalade (mM)

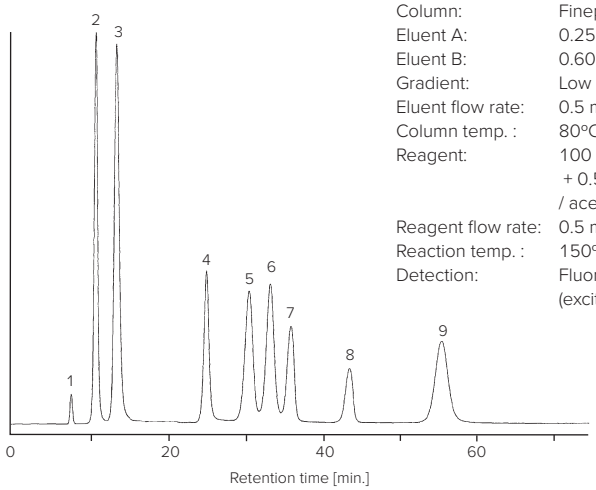
	Strawberry jam	Orange marmalade
Fructose	1.618	1.322
Glucose	2.804	2.609
Sucrose	2.565	2.251
Maltose	0.583	0.873

Fluorescence Detection via Post-Column Derivatization

Although RI detection is simple and universal, it sometimes lacks the sensitivity and selectivity required for low-concentration components or samples with closely eluting components. In such cases, sugars can be derivatized with a fluorescent reagent, such as guanidine in combination with metaperiodic acid, to significantly enhance both detection sensitivity and selectivity.

This section shows the HPLC analysis of sugars using fluorescence detection following post-column derivatization (a method where the sample is derivatized after elution from the column). An anion exchange column was used to achieve improved separation of structurally similar sugars with excellent reproducibility.

Figure 2 shows the chromatogram of a standard mixture of nine sugars. Figures 3 and 4 show the chromatogram of an extracted vegetable stew and an extracted condensed noodle soup, respectively. Each sugar component was well resolved with high sensitivity and reproducibility.



Conditions

Column:	Finepak GEL SA-121 (6.0 mmI.D. × 100 mmL)
Eluent A:	0.25 M borate buffer (pH 8.0)
Eluent B:	0.60 M borate buffer (pH 9.0)
Gradient:	Low pressure gradient elution
Eluent flow rate:	0.5 mL/min.
Column temp. :	80°C
Reagent:	100 mM borate + 50 mM guanidine hydrochloride + 0.5 mM sodium metaperiodate (pH 8.0, adjust with NaOH) / acetonitrile (80/20)
Reagent flow rate:	0.5 mL/min
Reaction temp. :	150°C
Detection:	Fluorescence detector (excitation: 310 nm, emission: 415 nm)

Figure 2. Chromatogram of a standard mixture of sugars

1: Sucrose, 2: Maltose, 3: Lactose, 4: Mannose, 5: Fructose, 6: Galactose, 7: Sorbitol, 8: Mannitol, 9: Glucose

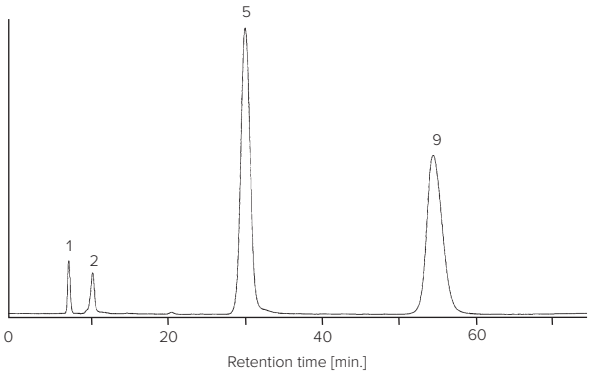


Figure 3. Chromatogram of extract of vegetable stew

Chromatographic conditions and peak numbers are the same as in Figure 2.

Preparation: The vegetable stew extract was filtered through a 0.45 µm membrane filter, then diluted 5 times with water and mixed. A sample volume of 20 µL was injected.

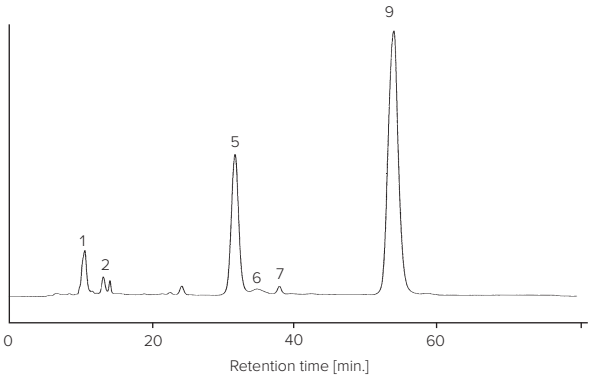


Figure 4. Chromatogram of condensed noodle soup

Chromatographic conditions and peak numbers are the same as in Figure 2.

Preparation: The condensed noodle soup was added to hot water (60°C), and ultrasonicated for 1 minute. After it was heated in a hot bath (65°C) for 5 minutes, it was left at room temperature for 30 minutes. Then, the supernatant (10 mL) was collected, and subject to centrifugal separation (4,000 rpm, 10 minutes). 6 % perchloric acid (1 mL) and chloroform (1 mL) was added to the obtained supernatant (1 mL) and stirred. Finally, the supernatant was filtered through a 0.45 µm membrane filter. A sample volume of 20 µL was injected.

Rapid separation of organic acids in beer using an ODS column (HPLC)

Organic acids in fermented food products are produced as a by-product of the fermentation process by lactic acid bacteria, Organic acids are metabolic by-products produced by lactic acid bacteria during the fermentation of food and beverages. These compounds significantly contribute to the taste of fermented products, such as sourness and deliciousness (umami). In addition, organic acids exhibit antioxidant and antibacterial activity, making them increasingly important for functional food research.

Ion exclusion chromatography has been the standard method for analyzing organic acids. The addition of a pH indicator, such as bromothymol blue (BTB), after the column can enhance selectivity by improving the distinction between target analytes and potential contaminants (Figure 1). While this method is effective across a wide range of organic acids with varying hydrophobicity, reversed-phase chromatography offers advantages in speed and sensitivity for low hydrophobicity organic acids. This section shows the separation of organic acids using an ODS (C18) column.

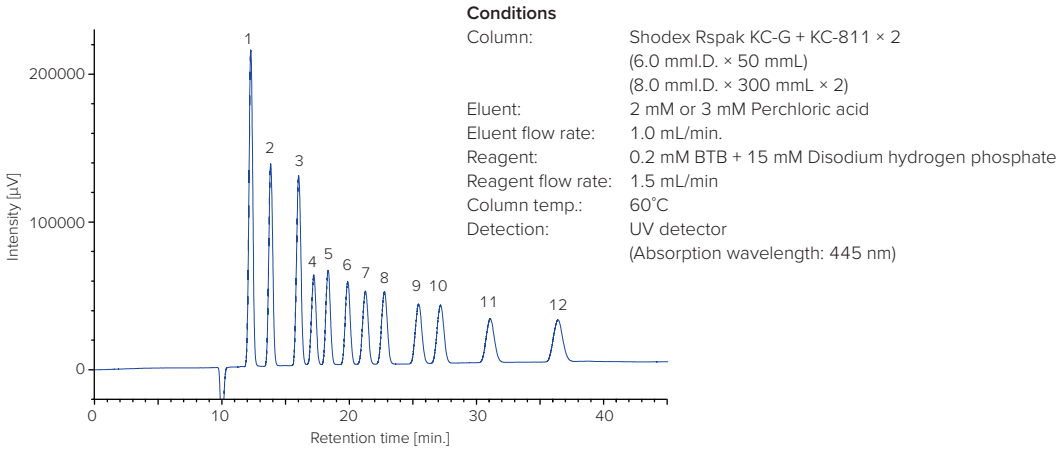
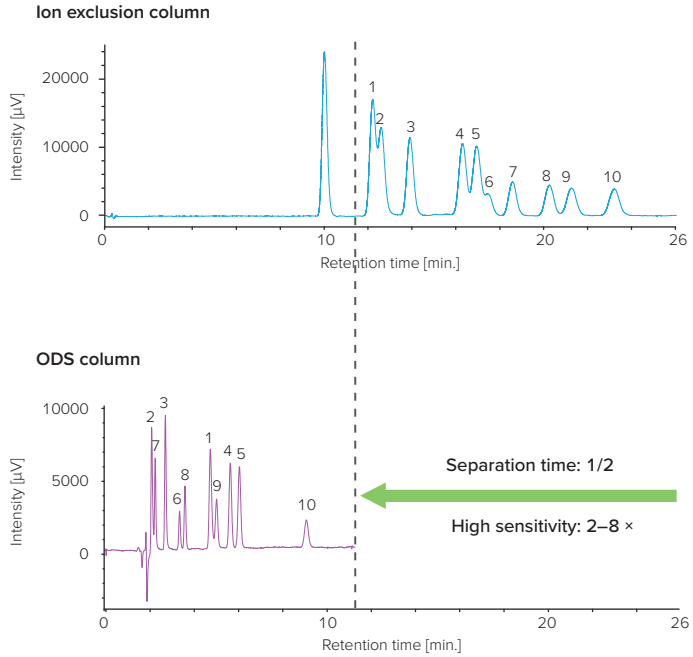


Figure 1. Chromatogram of a standard mixture of organic acids (by ion exclusion column)

1: Citric acid, 2: Malic acid, 3: Succinic acid, 4: Lactic acid, 5: Formic acid, 6: Acetic acid, 7: Pyroglutamic acid, 8: Propionic acid, 9: Isobutyric acid, 10: n-Butyric acid, 11: Isovaleric acid, 12: n-Valeric acid (5.0 mM each)

Analysis of Standard Organic Acids

Figure 2 shows the chromatograms of a standard mixture of ten organic acids analyzed using an ion exclusion and ODS column. The ODS column separated ten organic acids in approximately half the analysis time, improved peak resolution, and enhanced the sensitivity compared to the ion exclusion column.



Conditions (ion exclusion column)

Column:	Shodex Rspak KC-G + KC-811 × 2 (6.0 mmI.D. × 50 mmL) (8.0 mmI.D. × 300 mmL × 2)
Eluent:	2 mM or 3 mM Perchloric acid
Eluent flow rate:	1.0 mL/min.
Reagent:	0.2 mM BTB + 15 mM Disodium hydrogen phosphate
Reagent flow rate:	1.5 mL/min.
Column temp.:	60°C
Detection:	UV detector (absorption wavelength: 445 nm)

Conditions (ODS column)

Column:	InertSustain AQ-C18 (4.0 mmI.D. × 10 mmL, 5 μm) (4.6 mmI.D. × 150 mmL, 5 μm)
Eluent:	5 mM Perchloric acid
Eluent flow rate:	1.0 mL/min.
Reagent:	0.2 mM BTB + 15 mM Disodium hydrogen phosphate
Reagent flow rate:	1.0 mL/min.
Column temp.:	40°C
Detection:	UV detector (absorption wavelength: 445 nm)

Figure 2. Chromatograms of a standard mixture of organic acids (by ion exclusion column and ODS column)

1: Citric acid, 2: Tartaric acid, 3: Malic acid, 4: Succinic acid, 5: Fumaric acid, 6: Lactic acid, 7: Formic acid, 8: Acetic acid, 9: Pyroglutamic acid, 10: Propionic acid (0.5 mM each)

Quantitative Comparison of Organic Acids in Beer

Figure 3 shows the chromatograms of beer analyzed using an ion exclusion column and ODS column. Table 1 shows the quantitative values of six organic acids found in the beer sample. Both columns provided similar quantitative values, demonstrating that an ODS column offers the added benefits of faster analysis and enhanced sensitivity compared to the ion exclusion column, making it a reliable and efficient alternative for routine quality control or high-throughput analysis of organic acids in beer.

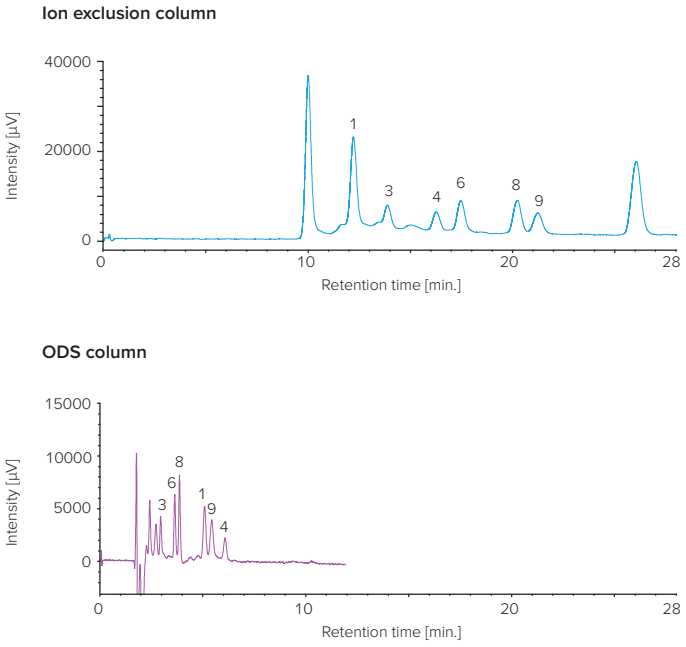


Table 1. Quantitative values of organic acids in beer (mM)

	Ion exclusion column	ODS column
Malic acid	0.19	0.17
Lactic acid	0.97	0.98
Acetic acid	0.83	0.95
Citric acid	0.58	0.49
Pyroglutamic acid	0.58	0.69
Succinic acid	0.17	0.16

Figure 3. Chromatograms of beer (by ion exclusion column and ODS column)

Chromatographic conditions and peak numbers are the same as in Figure 1.

Preparation: Beer was diluted twice with 1.5 mM perchloric acid, then filtered through a 0.45 μm membrane filter.

Rapid Separation of Nonvolatile Amines in Wine using OPA Derivatization (RHPLC)

Nonvolatile amines are produced during the microbial degradation of proteins and amino acids. Several nonvolatile amines, including histamine, are associated with food borne illnesses, making their accurate and efficient quantification critical for quality control in the food and beverage industry.

Traditionally, nonvolatile amines are analyzed using High-Performance Liquid Chromatography (HPLC) with dansyl chloride (DNS-Cl) derivatization. While this method is effective, it is also labor-intensive and time-consuming, involving extensive sample preparation and requiring a chromatographic separation time of around 40 minutes.

A more efficient alternative is pre-column derivatization using o-phthalaldehyde (OPA), which readily reacts with primary amines to form highly fluorescent derivatives, significantly reducing sample preparation time, shortening analysis time, and enabling more sensitive detection. This section shows a rapid HPLC (RHPLC) method for analyzing nonvolatile amines in wine using OPA derivatization.

Comparison of Derivatization Methods (DNS-Cl and OPA)

Figures 1 and 2 show the reaction mechanisms of DNS-Cl and OPA derivatization, respectively. DNS-Cl derivatization involves multiple labor-intensive steps, including sample preparation, reaction, extraction, concentration, and reconstitution, whereas OPA derivatization can be fully automated once the reagents are prepared by selecting the autosampler pre-column derivatization parameters. The OPA derivatization method can be completed in a quarter of the time required for DNS-Cl derivatization.

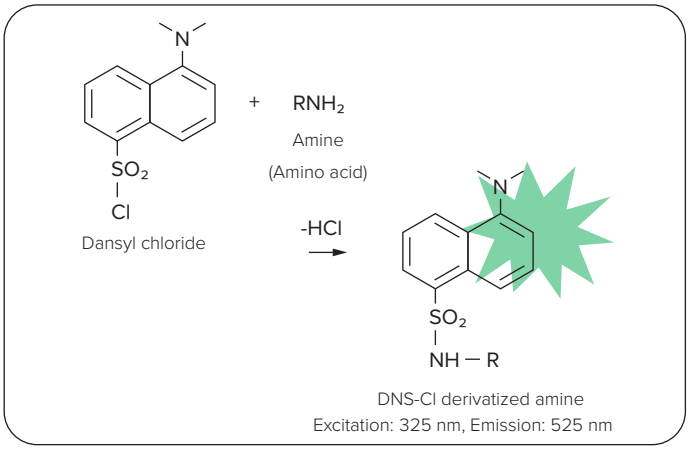


Figure 1. Reaction mechanism of DNS-Cl derivatization

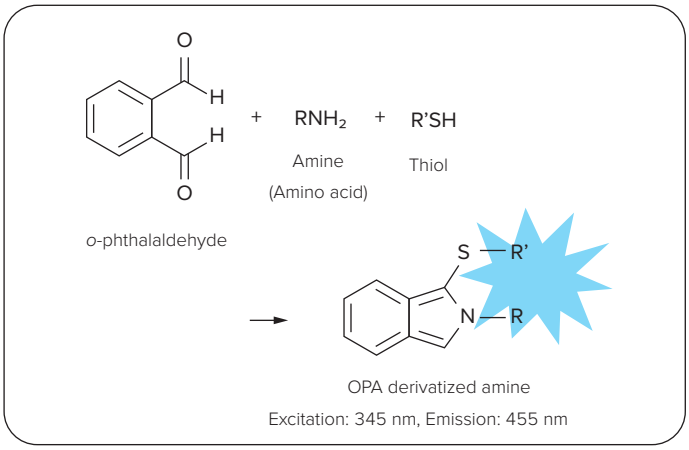
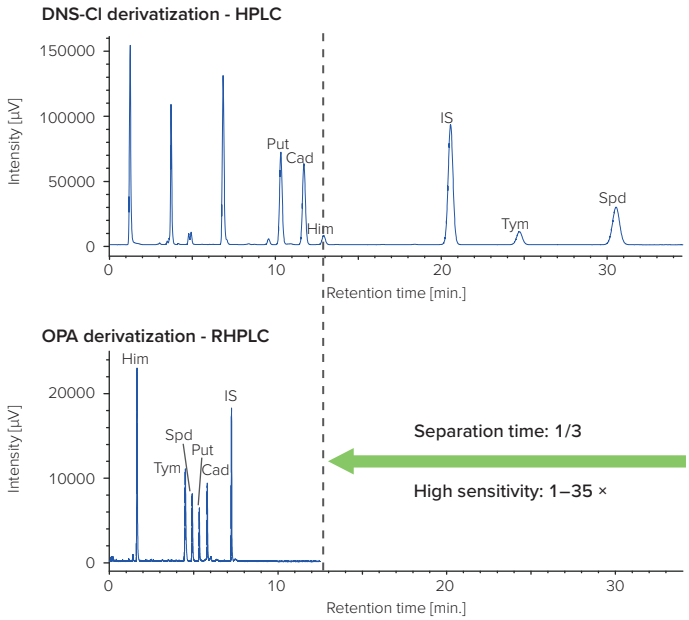


Figure 2. Reaction mechanism of OPA derivatization

Analysis of Standard Nonvolatile Amines

Figure 3 shows the chromatograms of a mixture of standard nonvolatile amines separated using HPLC and RHPLC methods. The RHPLC method separated six nonvolatile amines in one-third of the time, improved peak separation, and enhanced the sensitivity compared to the conventional HPLC method.



Conditions (HPLC)

Column:	Inertsil ODS-SP (4.6 mmI.D. × 250 mmL, 5 µm)
Eluent:	Water/Acetonitrile (35/65), Isocratic
Flow rate:	1.2 mL/min.
Column temp.:	40 °C
Detection:	Fluorescence detector (exciation: 325 nm, emission: 525 nm)

Conditions (RHPLC)

Column:	Sunshell C18 (2.1 mmI.D. × 50 mmL, 2.6 µm)
Eluent A:	1M Citrate buffer 3.5 mL in Water
Eluent B:	1M Citrate buffer 3.5 mL in water/acetonitrile/ethanol (5/4/1)
Gradient:	High pressure gradient elution
Flow rate:	1.0 mL/min.
Column temp.:	40 °C
Detection:	Fluorescence detector (excitation: 345 nm, emission: 455 nm)

Figure 3. Chromatograms of nonvolatile amines (by HPLC and RHPLC)

Put: Putrescine, Cad: Cadaverine, Him: Histamine, IS: 1,8-diaminooctane, Tym: Tyramine, Spd: Spermidine

Comparison of Quantitative Values of Nonvolatile Amines in Wine

Figure 4 shows the chromatogram of a wine sample separated using HPLC and RHPLC methods. Table 1 shows the quantitative values of five nonvolatile amines found in the wine sample. Both HPLC and RHPLC methods provided the same quantitative values, demonstrating that the faster RHPLC-OPA method offers a reliable and efficient alternative for routine analysis of nonvolatile amines in wines.

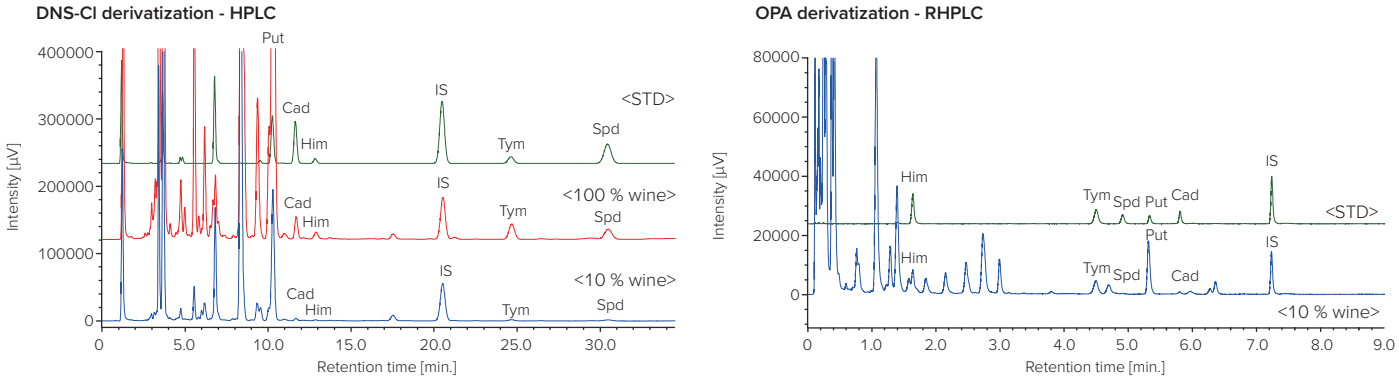


Figure 4. Chromatograms of wine (by HPLC and RHPLC)

Chromatographic conditions and peak numbers are the same as in Figure 3.

Preparation: Wine was diluted 10 times with water, then filtered through a 0.45 µm membrane filter

Table 1. Quantitative values of non-volatile amines in wine (µM)

	HPLC	UHPLC
Him	25.2	24.9
Tym	32.0	28.1
Cad	3.95	5.07
Put	226.4	203.1
Spd	4.13	4.11

Identification Test of Food Additives (FTIR)

When manufacturing or using food additives, each country enforces its own regulations governing the identification, purity, and quality of these substances. These regulations are strictly enforced to ensure that non-compliant additives are neither sold nor used. Both food and additive manufacturers are responsible for verifying that their products meet domestic regulatory standards, as well as the standards of any importing countries.

Many of these regulations include clearly defined test methods, and several specify the use of infrared (IR) spectrometry for additive identification. Typically, this involves comparing a sample's measured IR spectrum with either a reference spectrum or the spectrum of a certified reference standard. However, interpreting spectral data often requires a skilled operator.

To support objective and reproducible identification, spectral comparison algorithms can be employed. These algorithms automate the comparison process and reduce reliance on subjective interpretation. Common algorithms include:

Euclidean Distance Calculates the sum of the squared absorbance differences at each wavenumber. Suitable for comparing spectra with broad absorption bands.

Euclidean Distance After Differentiation Similar to the basic Euclidean distance but applied after differentiating the spectra. Effective for handling baseline drift and comparing spectra with sharp peaks.

Correlation Coefficient Measures the degree of correlation between two spectra. Useful for comparing spectra that contain noise.

This article presents examples of p-methylacetophenone (used as a flavoring agent) and aspartame (used as a sweetener), analyzed according to the official test methods outlined in Japan's Specifications and Standards for Food Additives.

JASCO FTIR systems provide objective product evaluation (free from operator bias) and eliminate the need for in-depth knowledge of IR spectral data analysis. These systems offer a range of functions that make product evaluation both easy and routine.

- Comprehensive Database of IR Spectra of Reference Standards
- Statistical Comparison of IR Spectra Between Sample and Reference
- Seven Different Comparison Algorithms Tailored for Various Types of Spectral Data
- Objective Evaluation Using Pass/Fail Judgement Criteria
- 21 CFR Part 11 Compliance Available for Regulatory Adherence

Identification Test of *p*-methylacetophenone

A liquid sample was placed between two KBr windows and measured in transmission using an FTIR spectrometer (Liquid Film Method). Figure 1 displays the results.

According to the regulations, the spectrum of the sample should exhibit similar absorption intensities at corresponding wavenumbers when compared to a standard reference spectrum. By selecting an appropriate comparison algorithm, and depending on the specific context, it is possible to quantify the similarity between the sample spectrum and a database of reference spectra. This allows for the detection of subtle differences between spectra, even when the spectra may appear visually similar to the user.

"Euclidean distance after differentiation" was used as the comparison algorithm. In this method, a lower score indicates higher similarity, with the score approaching 0 when the similarity is high. By differentiating the spectra, any baseline slope is offset, allowing for more accurate comparisons between the two spectra.

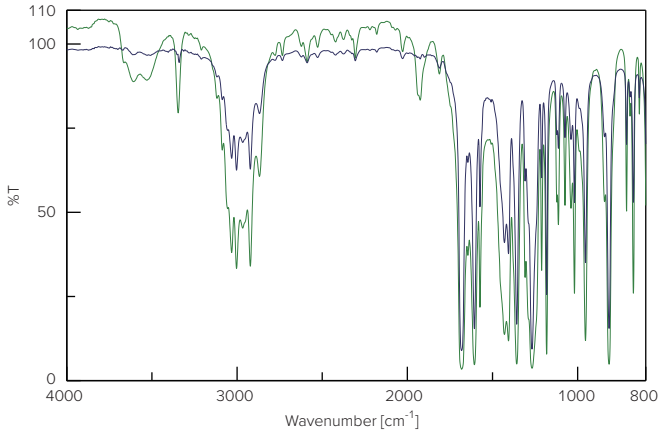


Figure 1. IR spectra of *p*-methylacetophenone
(green: sample, blue: reference standard)

To establish clear criteria, several samples were measured, and the Euclidean distances between each sample and its reference were calculated in advance. Based on these calculations, the acceptance criteria were set to a threshold value of 60.

The score of the measurement result shown in Figure 1 is 37.29, which is below the threshold and therefore qualifies as a "PASS". This objective evaluation ensures that the result meets the required criteria.

Identification Test of Aspartame

A paste sample was prepared by mixing the triturated sample with liquid paraffin. The paste was then sandwiched between two KBr plates and measured using an FTIR spectrometer (Paste Method). Figure 2 presents the resulting spectrum.

According to the regulation, the spectrum should exhibit absorption bands at specific wavenumbers. This test can be performed objectively by setting the appropriate wavenumbers and tolerances within the software, ensuring accurate and consistent results.

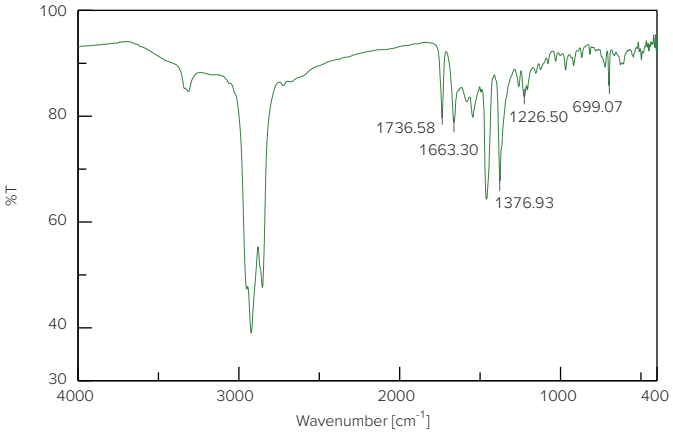


Figure 2. IR spectrum of aspartame

Table 1. Test result

Peak wavenumber (tolerance)	Measurement value	Pass/Fail
1737.00 cm^{-1} (5.00 cm^{-1})	1736.58 cm^{-1}	Pass
1666.00 cm^{-1} (5.00 cm^{-1})	1663.30 cm^{-1}	Pass
1379.00 cm^{-1} (5.00 cm^{-1})	1376.93 cm^{-1}	Pass
1227.00 cm^{-1} (5.00 cm^{-1})	1226.50 cm^{-1}	Pass
699.00 cm^{-1} (5.00 cm^{-1})	699.07 cm^{-1}	Pass

Analysis of Preservatives and Sweeteners (HPLC)

Food additives play a vital role in enhancing the sensory qualities, shelf life, and nutritional value of processed foods. Food additives fall into several different categories: 1) Flavor enhancers, such as sweeteners, colorants, and spices, to improve the taste, appearance, and/or aroma, 2) Preservatives to prevent spoilage, discoloration, 3) Nutritional additives to fortify food with essential nutrients, such as vitamins and minerals.

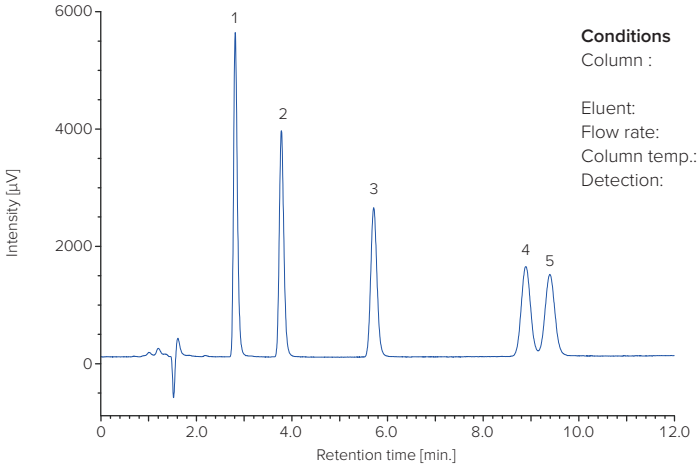
While food additives offer significant benefits, excessive consumption can have adverse health effects. Regulatory agencies around the world, such as the U.S. Food and Drug Administration (FDA), rigorously evaluate and monitor food additives to ensure consumer safety. The FDA considers the following when evaluating the safety of a substance to be approved as a food additive:1) the chemical composition and physical properties of the substance, 2) estimated daily intake levels, 3) potential short- and long-term health effects, 4) additional safety factors and toxicological data. This evaluation leads to a legally approved safe usage level that includes a built-in safety margin to account for uncertainty in human consumption. Food manufacturers must ensure their products comply not only with domestic regulations but also with the regulations of countries to which they export.

In Japan, food additives are regulated under the Food Sanitation Act, which outlines specifications and standards for their manufacture, sale, and use. In addition, analytical methods applicable to each food additive are published for compliance testing, many of which specify High-Performance Liquid Chromatography (HPLC) as the method for the analysis of food additives. This section shows the HPLC-based analysis of preservatives and sweeteners according to the methods published by the Japanese authorities.

Analysis of *p*-hydroxybenzoate esters

p-hydroxybenzoate esters, also known as parabens, are widely used food additives as preservatives due to their broad-spectrum antimicrobial activity. Figure 1 shows a chromatogram of a standard mixture containing five different p-hydroxybenzoate esters. All five components were separated with good resolution in under 10 minutes.

*U.S. FOOD & DRUG ADMINISTRATION (2010, April), *Overview of Food Ingredients, Additives & Colors*, https://www.fda.gov/food/food-ingredients-packaging/overview-food-ingredients-additives-colors



Conditions

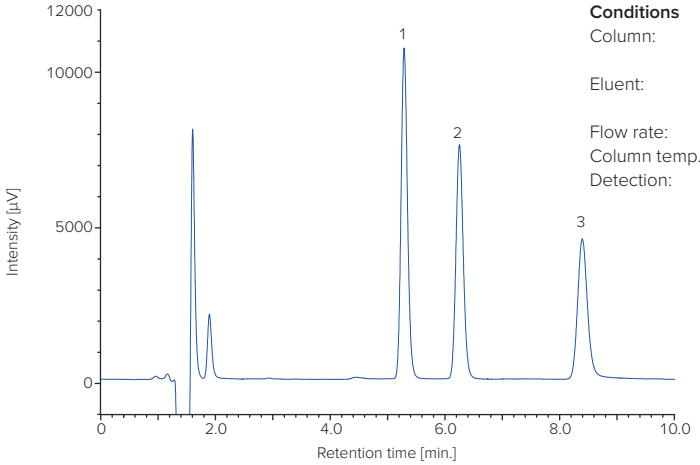
Column :	CrestPak C18S (4.6 mmI.D. × 150 mmL, 5 µm)
Eluent:	Acetonitrile/5 mmol/L Citrate buffer (pH 4.1) (60/40)
Flow rate:	1.0 mL/min.
Column temp.:	40 °C
Detection:	UV detector (absorption wavelength: 270 nm)

Figure 1. Chromatogram of standard *p*-hydroxybenzoate esters

1: Methyl *p*-hydroxybenzoate, 2: Ethyl *p*-hydroxybenzoate, 3: Propyl *p*-hydroxybenzoate,
4: Isobutyl *p*-hydroxybenzoate, 5: Butyl *p*-hydroxybenzoate

Analysis of Benzoic Acid, Sorbic Acid, and Dehydroacetic Acid

Benzoic acid, sorbic acid, and dehydroacetic acid are common preservatives used as food additives to inhibit microbial growth. Figure 2 shows the chromatogram of a standard mixture containing benzoic acid, sorbic acid, and dehydroacetic acid. All three components were separated with good resolution in under 9 minutes.



Conditions

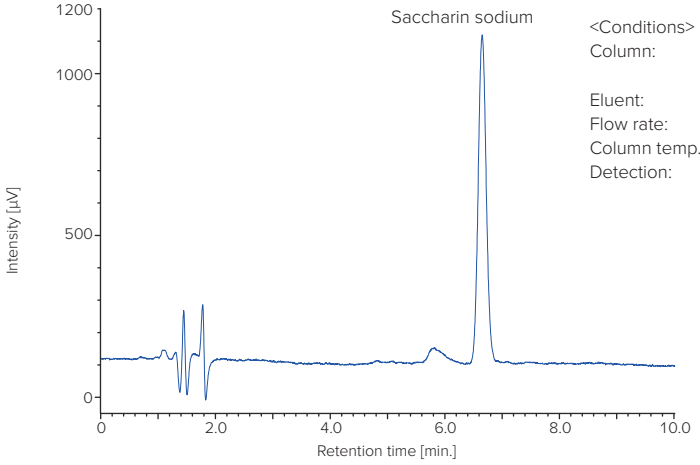
Column:	CrestPak C18S (4.6 mmI.D. × 150 mmL, 5 µm)
Eluent:	5 mmol/L Citrate buffer (pH 4.1) / Acetonitrile / Methanol (70/20/10)
Flow rate:	1.0 mL/min.
Column temp.:	40 °C
Detection:	UV detector (absorption wavelength: 230 nm)

Figure 2. Chromatogram of standard mixture (benzoic acid, sorbic acid and dehydroacetic acid)

1: Benzoic acid, 2: Sorbic acid, 3: Dehydroacetic acid

Analysis of Saccharin Sodium

Saccharin sodium is a food additive used as sweetener used as a food additive in beverages and processed foods. Figure 3 shows the chromatogram of saccharin sodium standard.



<Conditions>

Column:	CrestPak C18S (4.6 mmI.D. × 150 mmL, 5 µm)
Eluent:	0.05 mol/L Phosphate buffer (pH 6.9)/Methanol (90/10)
Flow rate:	1.0 mL/min
Column temp.:	40 °C
Detection:	UV detector (absorption wavelength: 230 nm)

Figure 3. Chromatogram of saccharin sodium

Rapid Separation of Food Additives in a Functional Beverage (UHPLC)

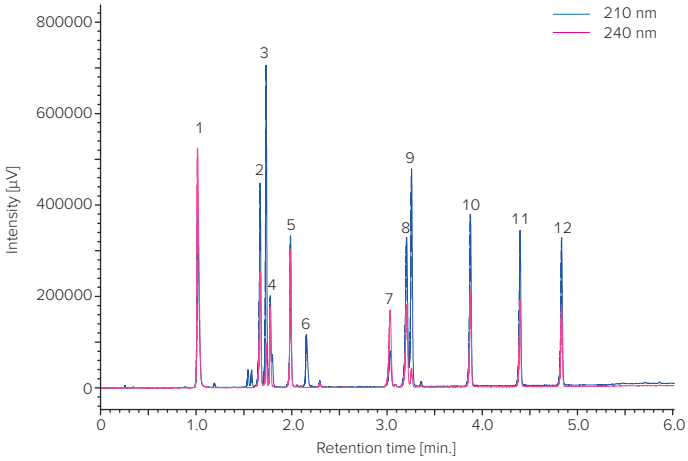
High-Performance Liquid Chromatography (HPLC) is a widely used technique for analyzing food additives due to its reliability and ease of use; however, increasing demands for higher throughput have created the need for faster analysis times than those achievable with conventional HPLC systems. The optimal absorption wavelength varies depending on the components of interest, often making it difficult to monitor all components in a mixture at a single wavelength, particularly in complex mixtures with multiple components.

UHPLC, combined with a Photometric Diode Array (PDA) detector, provides a powerful solution for fast and efficient multi-component analysis. UHPLC significantly shortens run times while maintaining excellent resolution compared to conventional HPLC. A PDA detector, capable of high-speed spectral data acquisition up to 100 Hz, allows for simultaneous monitoring of each component at its optimal absorption wavelength, enhancing detection sensitivity. Furthermore, PDA detection enables spectral matching of each component by comparing its UV spectrum to that of a known standard reference material, ensuring accurate qualitative analysis even in complex unknown samples.

This section shows the results of a UHPLC system with a PDA detector for the analysis of food additives in a functional beverage.

Analysis of Standard Food Additives

Figures 1 and 2 on the following page show the chromatogram and PDA contour plot for a mixture of 12 standard food additives, respectively. All components were baseline separated in under 5 minutes, illustrating the high efficiency of the UHPLC method.



Conditions

Column:	ZORBAX Eclipse Plus C18 (3 mmI.D. x 50 mmL, 1.8 µm)
Eluent A:	0.3% Phosphoric acid solution
Eluent B:	Acetonitrile
Gradient:	High pressure gradient elution
Flow rate:	0.8 mL/min.
Column temp.:	40 °C
Detection:	PDA detector (absorption wavelength: 200 to 650 nm)

Figure 1. Chromatogram of standard food additives

1: Acesulfame K (0.2 mg/mL), 2: p-Hydroxybenzoic acid (0.05 mg/mL), 3: Caffeine (0.05 mg/mL), 4: Vitamin B2 sodium phosphate(Riboflavin sodium phosphate) (0.1 mg/mL), 5: Vitamin B2(Riboflavin) (0.1 mg/mL), 6: Aspartame (0.1 mg/mL), 7: Benzoic acid (0.05 mg/mL), 8: Methyl p-hydroxybenzoate (0.05 mg/mL), 9: Propyl gallate (0.05 mg/mL), 10: Ethyl p-hydroxybenzoate (0.05 mg/mL), 11: Propyl p-hydroxybenzoate (0.05 mg/mL), 12: Butyl p-hydroxybenzoate (0.05 mg/mL)

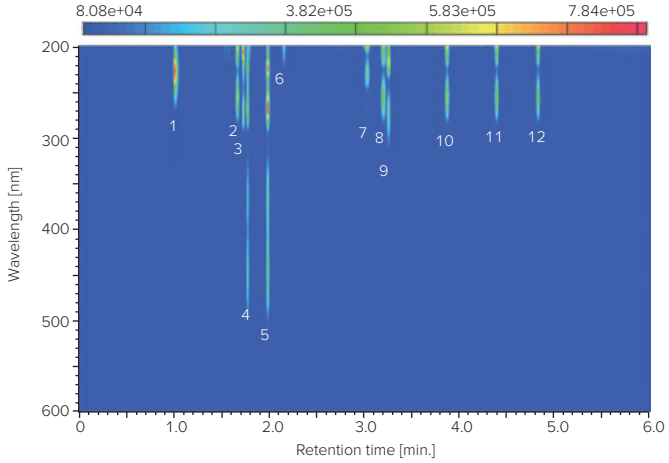


Figure 2. Chromatogram of standard food additives (contour plot)

Chromatographic conditions and peak numbers are the same as in Figure 1.

Analysis of Food Additives in a Functional Beverage

Figures 3 and 4 show the chromatogram and the PDA contour plot of a commercial functional beverage, respectively. Figure 5 shows the chromatograms at four different wavelengths, demonstrating the utility of PDA detection in capturing the full spectral behavior of individual compounds within a single run.

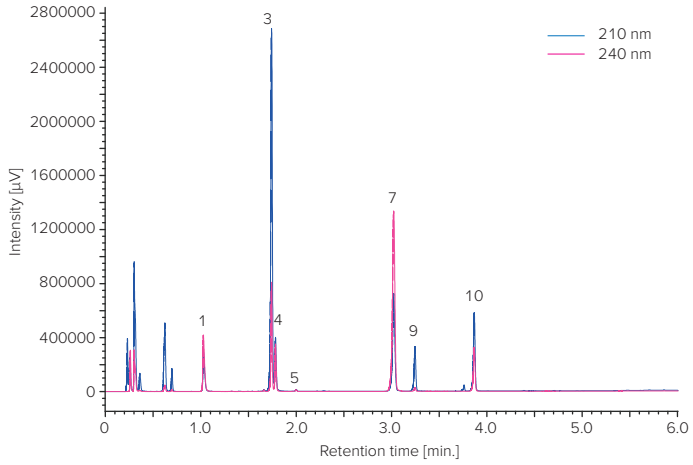


Figure 3. Chromatogram of a functional beverage

Chromatographic conditions and peak numbers are the same as in Figure 1.

Preparation: Functional beverage was filtrated through 0.2 µm membrane filter.

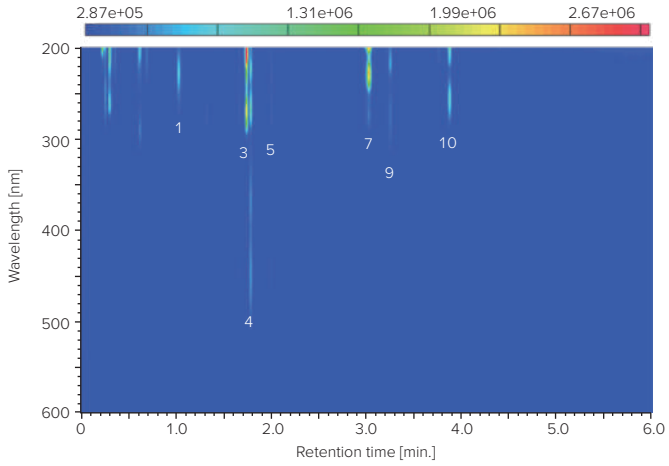


Figure 4. Chromatogram of a functional beverage (contour plot)

Chromatographic conditions and peak numbers are the same as in Figure 1.

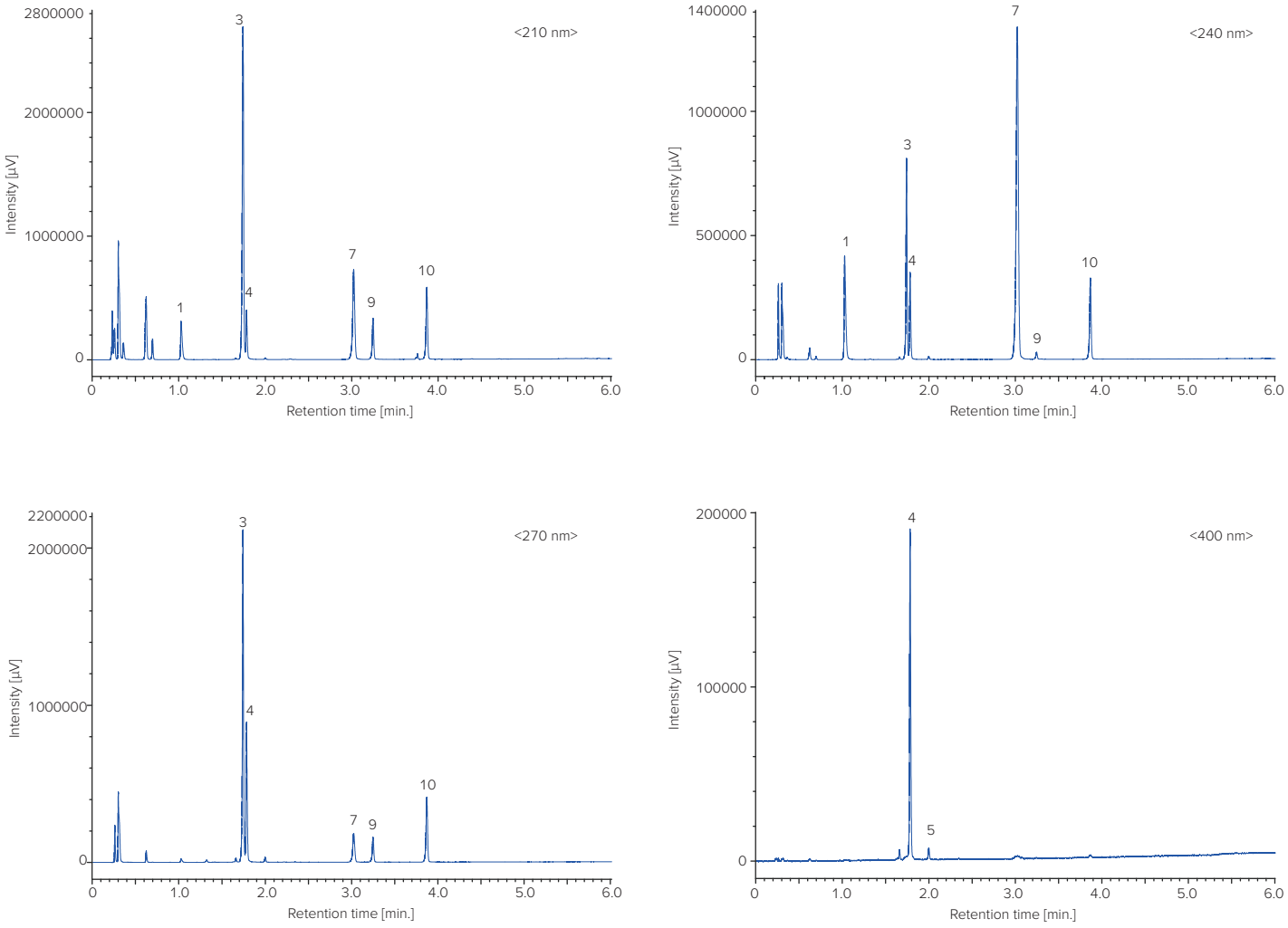


Figure 5. Chromatograms of a functional beverage at four different wavelengths (210 nm, 240 nm, 270 nm, 400 nm)

Chromatographic conditions and peak numbers are the same as in Figure 1.

Combined Analysis of Food Packaging Films (IR and Raman Microscopy)

Packaging materials play a crucial role in preserving the overall quality and extending the shelf life of food, ensuring freshness and safety for consumption. Defects in packaging films can lead to accelerated food deterioration, creating health risks and potentially resulting in product recalls. As a result, there is ongoing development in packaging materials, along with strict quality control measures throughout the manufacturing process, guided by the principles of Hazard Analysis and Critical Control Point (HACCP).

While various materials—such as metal, glass, and paper—are used for packaging depending on the application, plastics are particularly popular for food packaging due to their high safety margins and hygienic properties.

IR and Raman microscopy are powerful techniques used to assess the physical and chemical properties of packaging films, enabling the identification of defects and impurities. These two techniques are complementary, and when combined, they provide a more comprehensive analysis of the sample. Additionally, chemometrics applied to spectral data enhances the extraction of detailed component information. Visualizing the component distribution helps identify contamination and offers valuable insights that can drive innovations in research and development.

This article discusses the evaluation of food packaging films using these advanced techniques.

Combined Analysis of Multi-Layer Film

A cross-section of a multi-layer film was prepared and measured using both IR and Raman microscopy at the exact same location. Figure 1 shows the observed image of the cross-section, while Figures 2 and 3 display the principal component spectra calculated using Multivariate Curve Resolution (MCR). False-color maps of the chemical distribution were created using the scores from the principal component spectra.

By analyzing both the principal component spectra and the chemical images, the distribution of key materials such as polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET) was clearly visualized. The IR microscope specifically detected cellulose, which exhibits strong infrared activity, while the Raman microscope identified titanium oxide (TiO2) as a very thin layer (several micrometers thick).

Given that cellulose is not typically used in the manufacture of this film, its presence suggests a high likelihood of foreign material contamination. By combining data from both techniques, complementary information was obtained, enhancing the overall analysis of the film's structure and composition.

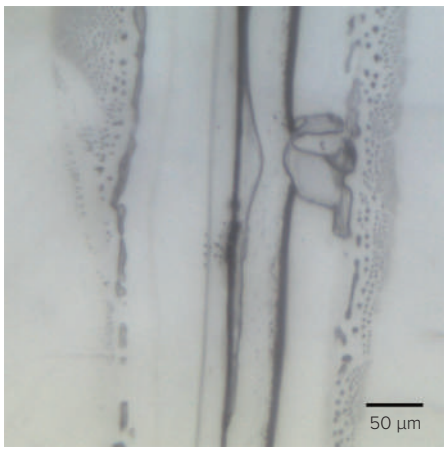


Figure 1. Observation view

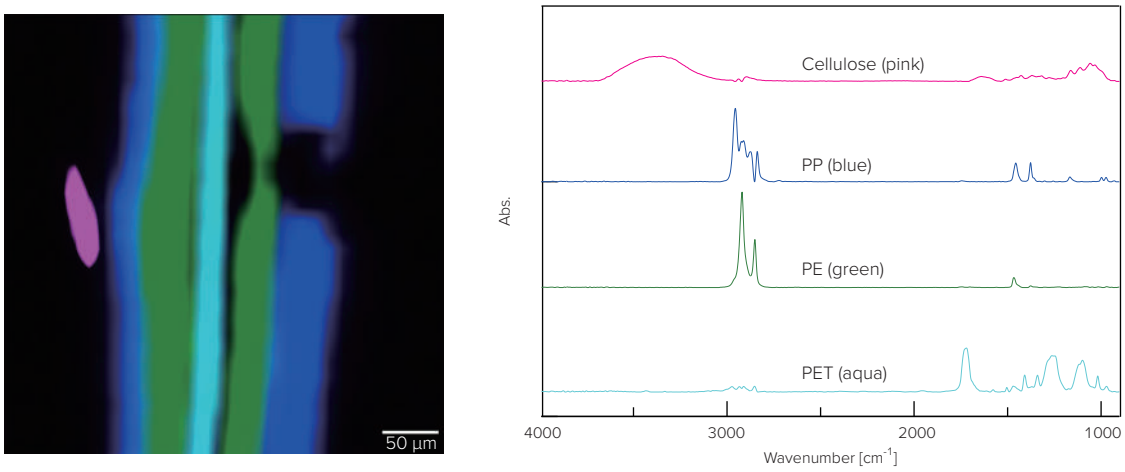


Figure 2. Principal component spectra (right) and chemical image (left) (IR microscope)

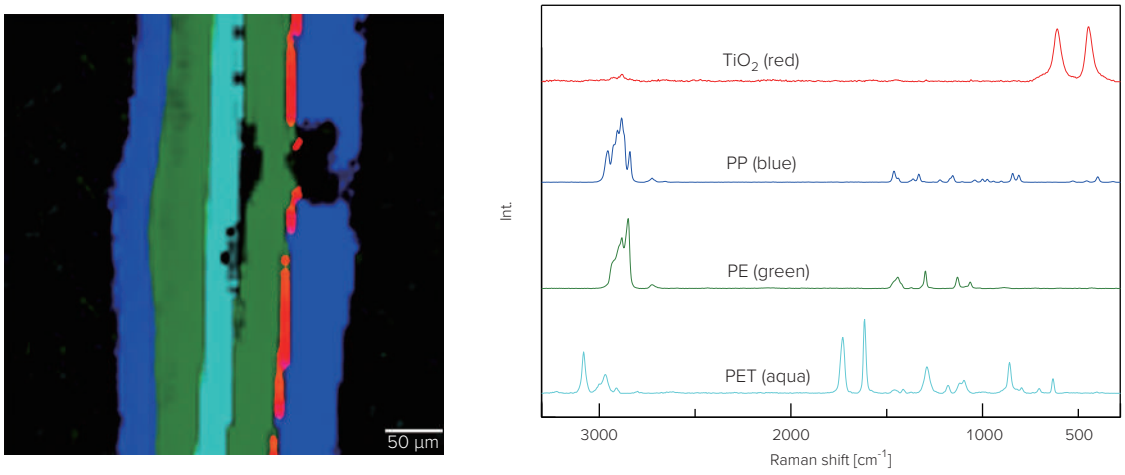
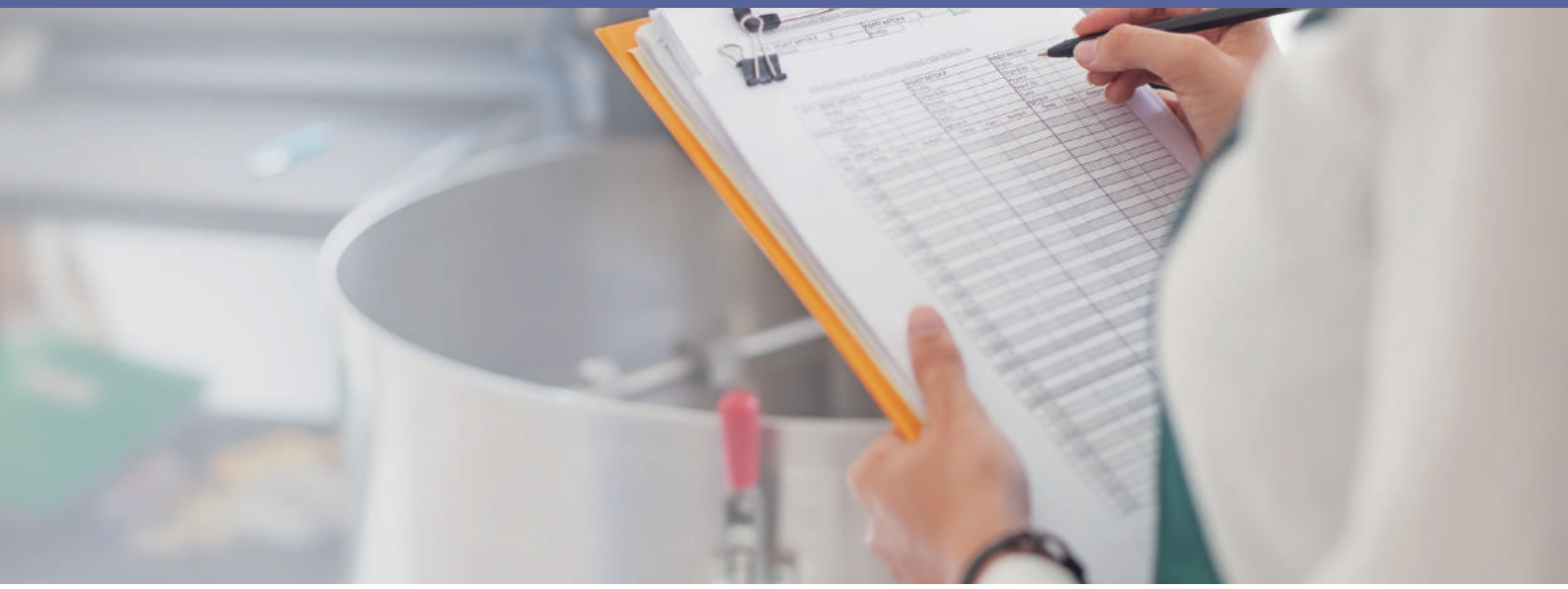


Figure 3. Principal component spectra (right) and chemical image (left) (Raman microscope)

JASCO has developed the IQ Frame, an innovative accessory that enables Infrared and Raman spectroscopy to be measured at precisely the same location, facilitating quick and convenient comparison. This accessory is particularly useful for microscopic analysis across different laboratories, re-measuring samples that have been removed and replaced on the stage, and performing combined IR and Raman analysis.



Combined Analysis of Impurities in Food (IR and Raman Microscopy)

Recalls due to contamination in food products are a global concern, with authorities publicly disclosing these incidents and mandating recalls. Contamination can occur for various reasons, including the presence of rubber and resins from deteriorating components in production equipment or human error. Therefore, understanding and identifying the causes of contamination is essential for ensuring the safe manufacture of food products.

In recent years, some countries have imposed hygiene control regulations under HACCP (Hazard Analysis and Critical Control Point), leading to stricter quality control practices and a reduction in product recalls.

IR and Raman microscopy are powerful tools for evaluating the physical and chemical properties of impurities found in food products and packaging materials. These techniques are complementary, and obtaining spectra from both provides synergistic and more detailed information. Additionally, applying chemometrics to spectral data allows for the extraction of underlying component information, which can serve as evidence to pinpoint the causes of product defects.

This article demonstrates the evaluation of plastic particles using IR and Raman microscopy.

Analysis of Plastic Particles using IR Microscopy

A sample sprayed with plastic particles was analyzed using an IR microscope. Figure 1 shows the observed image, while Figure 2 displays the principal component spectra calculated using Multivariate Curve Resolution (MCR), along with the chemical images created from the scores of these spectra.

The analysis revealed the presence of protein, as well as four distinct types of plastic: polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and polystyrene (PS).

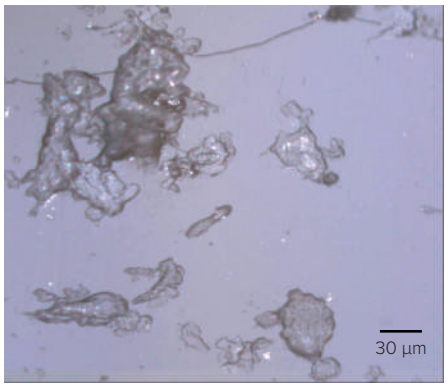


Figure 1. Observation view

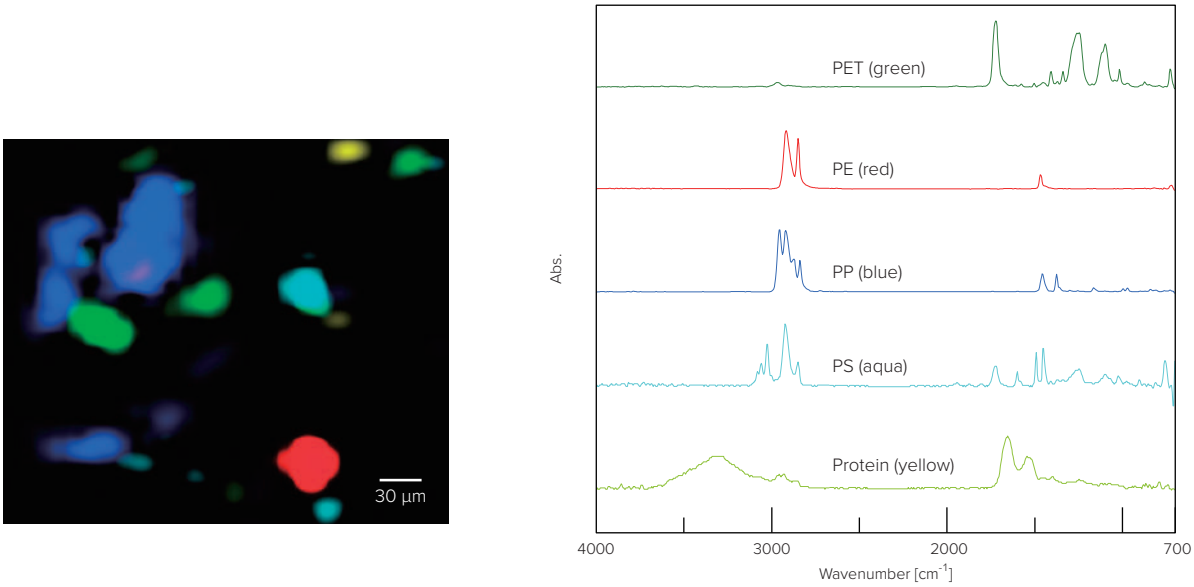


Figure 2. Principal component spectra (right) and chemical image (left) (IR)

Analysis of Plastic Particles using Raman Microscopy

Using the IQ Frame, Raman measurements were performed at exactly the same position as the previous IR microscope analysis. Figure 3 shows the principal component spectra calculated using Multivariate Curve Resolution (MCR), along with the chemical images constructed based on the peak heights of key bands.

The analysis confirmed the presence of carbon in addition to the four plastics previously identified using IR spectroscopy: polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and polystyrene (PS). While the IR microscope detected protein, the Raman microscope was able to selectively identify carbon. This result highlights the complementary strengths of the two techniques: Raman spectroscopy offers high sensitivity for carbon but low detection sensitivity for protein, making it ideal for carbon analysis.

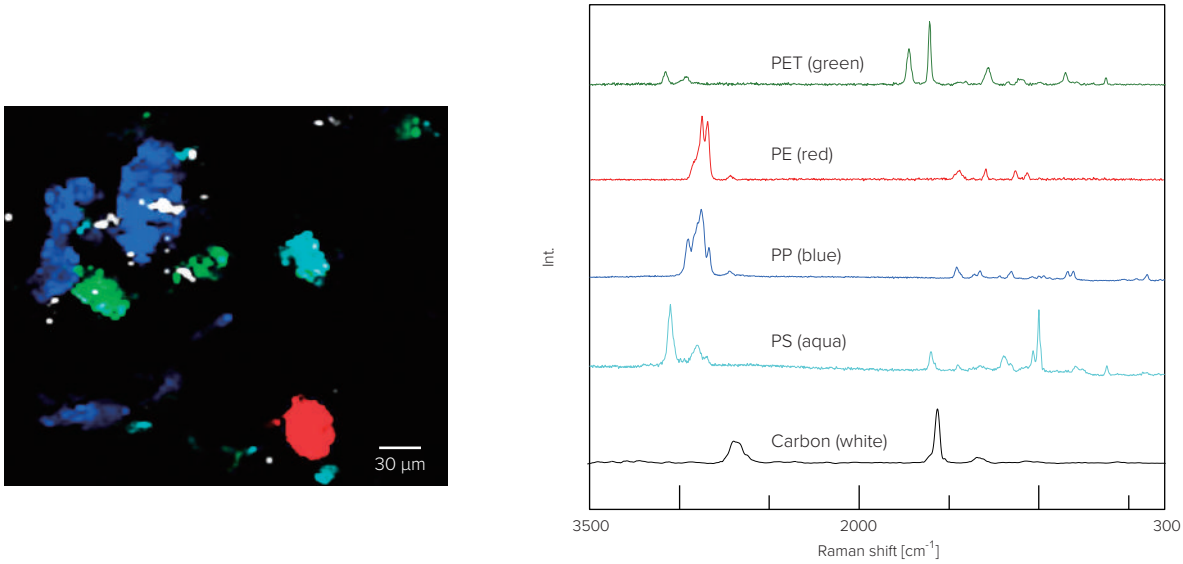


Figure 3. Principal component spectra (right) and chemical image (left) (Raman)

Overlay of Imaging Results (IR and Raman)

The imaging results of the plastic particle samples are shown in Figures 2 and 3. While only five components could be detected by either IR or Raman spectroscopy, the combined analysis revealed six distinct components.

To visualize the complete distribution of the sample, the imaging data from both techniques were overlaid (Figure 4). This overlay shows the protein detected by IR, along with the five plastics—polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS)—and the carbon identified using Raman.

These results highlight the synergy between IR and Raman spectroscopy, demonstrating the advantages of combined measurements over relying on a single analytical technique.

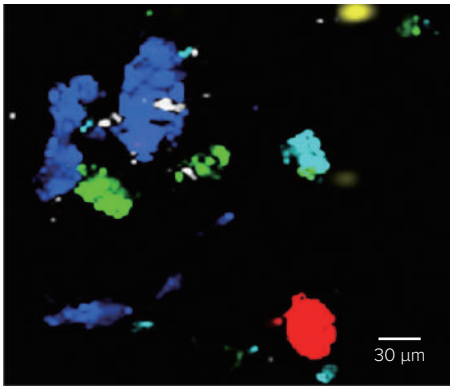


Figure 4. Overlaid image of IR and Raman

Time Estimation of Pest Contamination (Circular Dichroism)

Pest contamination can occur at any stage – from farm to table. When contamination is detected, manufacturers must quickly identify when and how pests were introduced into the food and take immediate corrective action during the product processing.

One effective method for determining the timing of pest contamination is to monitor the denaturation of proteins originating from the pest. By analyzing how the higher-order structure of these proteins changes irreversibly during heating, the timing of contamination in heat-treated food can be estimated. This can be achieved by assessing whether protein denaturation has occurred (see Figure 1).

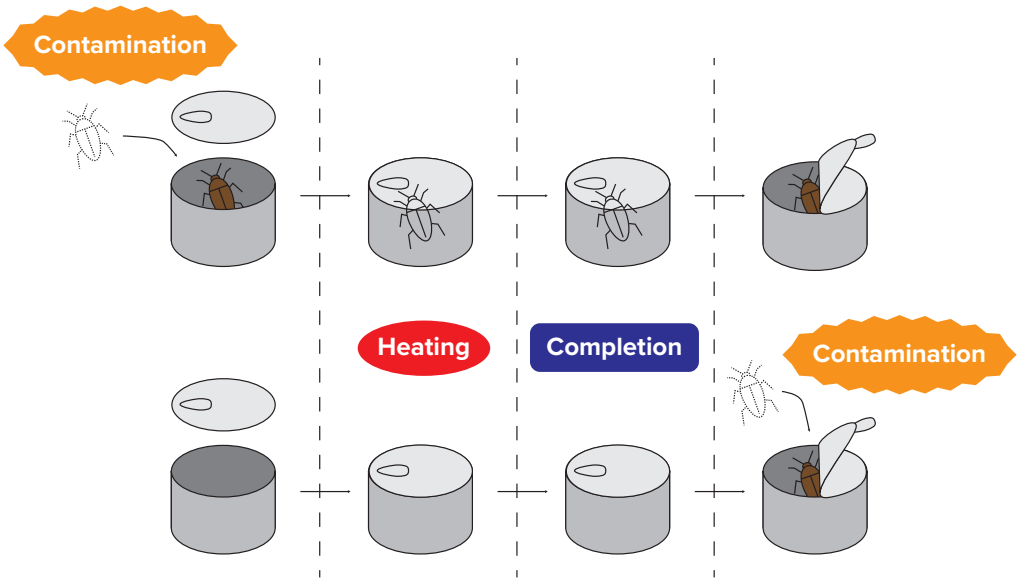


Figure 1. Time estimation of pest contamination

(Top: contamination during the food processing, Bottom: contamination after food processing)

Since CD spectroscopy can effectively detect changes in the higher-order structure of proteins extracted from pest samples, it serves as a valuable tool for estimating the timing of contamination.

This application note demonstrates how CD spectroscopy can provide critical insights into identifying the sources of pest contamination.

CD Spectrum Changes of Pest Femur Extract

Figure 2 presents the CD spectra of a pest femur extract and a sample of commercially obtained myosin, both before and after heating. The spectra from both samples are closely correlated, suggesting a significant concentration of myosin in the pest femur extract.

Figure 3 displays the results of the secondary structure estimation derived from the CD spectra, confirming that the ratio of secondary structures changed during the heat-treatment process.

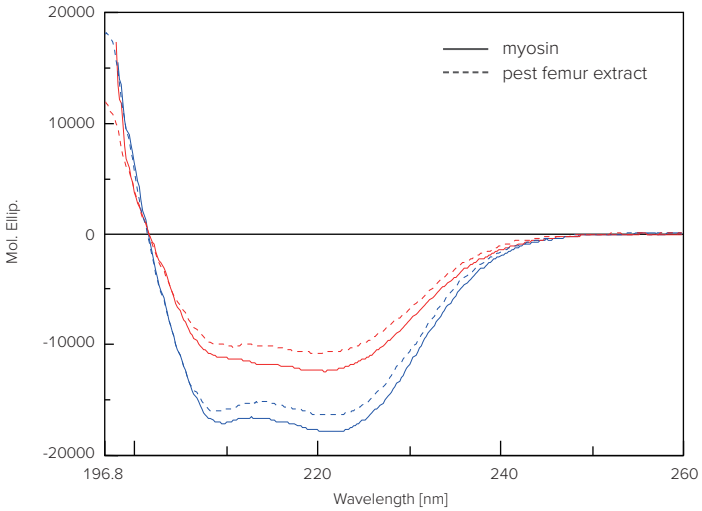


Figure 2. CD spectrum changes (blue: non-heating, red: heating)

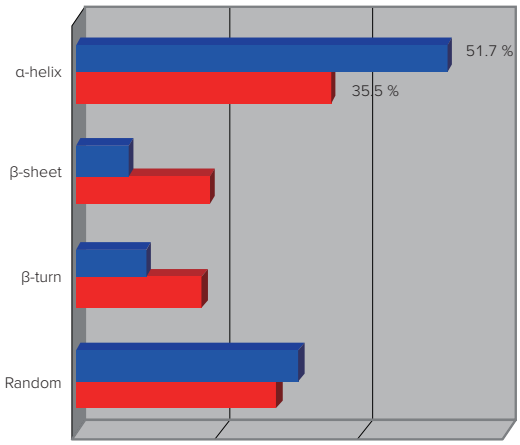


Figure 3. Secondary structure analysis (blue: non-heating, red: heating)

Denaturation Temperature of Myosin

Figure 4 presents the results of monitoring the structure of myosin during thermal ramping. The denaturation temperature of myosin was calculated to be 46.2°C, which indicates that analyzing changes in secondary structure can help estimate whether contamination occurred before or after heat-treatment.

If the heat-treatment temperature exceeds 46.2°C and the pest sample has not undergone denaturation, it suggests that the pest contamination occurred after heat-treatment, once the manufacturing process was completed.

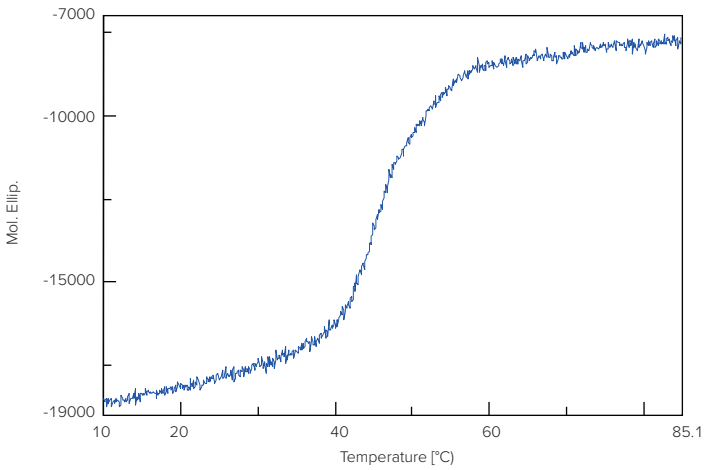


Figure 4. Temperature monitoring of myosin

Since the myosin structure is conserved across many species, this test can be applied to a broad range of pest types. Additionally, with a denaturation temperature of 46.2°C, it can be used to assess manufacturing processes across various sterilizing temperatures. CD spectroscopy analysis of myosin could be a valuable tool for forensic testing in heat-treated foods, such as canned foods and retort pouches.

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