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SHIMADZU
Excellence in Science

Shimadzu Analysis Guidebook

Food Product Analyses

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1. Food Product Components

1.1 Analysis of Fatty Acids in Fish (1) - GC/MS/MS

■Explanation

While some fatty acids, such as the n-3 fatty acids EPA and DHA, are beneficial to human health because they lower the amount of blood-borne neutral fat, too much intake of saturated fatty acids raises the risk of some diseases. For this reason, there is a need for the batch analysis of these fatty acids in the life sciences and food engineering sectors. Despite requiring methylation, GC-MS has gained attention because of its suitability for multicomponent batch analyses. In fatty acid analyses utilizing GC-MS, the EI (electron ionization) method is used for ionization. With the EI method, there are many types of fragment ions, making it easy to select an *m/z* to enable separation by mass from impurities. However, because of the large number of fragment ions, the sensitivity of the individual ions is reduced, making it difficult to detect trace quantities of fatty acids. In contrast, with the PCI (positive chemical ionization) method, protonated molecular ions can be detected, from which molecular weight data can be obtained. Since there is only a small number of fragment ion types, the sensitivity is increased. This means, however, that the ion types that can be selected for monitoring are limited and there may not be any ions that can be separated by mass from impurities. Here we introduce the results of an investigation of separation from impurities based on the EISIM, PCI-SIM, EI-MRM, and PCI-MRM methods.

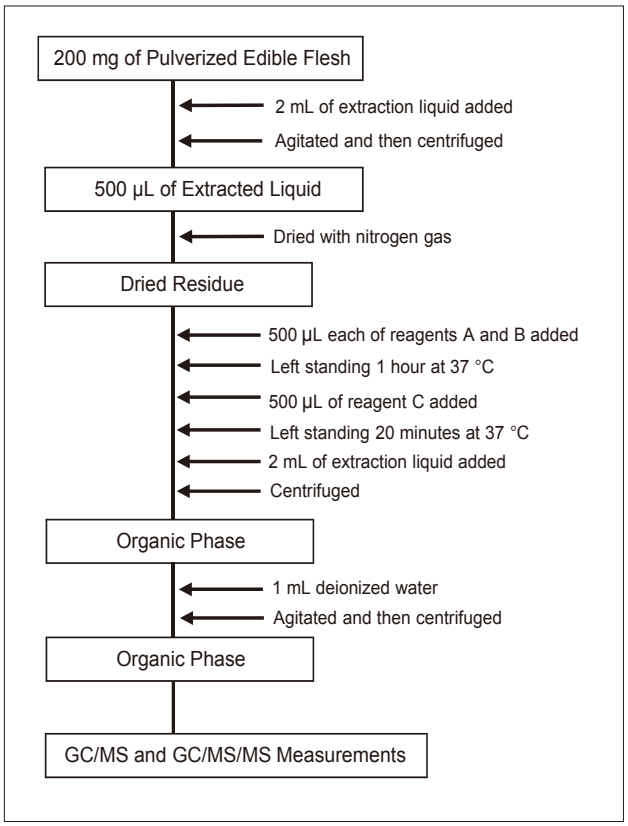


Fig. 1.1.1 Pretreatment of Saury

■Pretreatment Method

Saury (fish) was used to investigate the separation from impurities in each analysis mode. The fatty acid methylation kit (P/N: 06482) sold by Nacalai Tesque was utilized for the pretreatment. The pretreatment method is shown in Fig. 1.1.1. The edible flesh from the saury was collected and pulverized with a mill, after which 200 mg was measured out. After adding 2 mL of the extraction liquid and agitating, the mixture was centrifuged, and 500 µL of extracted liquid was obtained. The extracted liquid was dried under a nitrogen flow, and 500 µL each of reagents A and B were added. After leaving the mixture to stand for 1 hour at 37 °C, 500 µL of reagent C was added, and it was left to stand at 37 °C for a further 20 minutes. Afterward, 2 mL of the extraction liquid was added, and after centrifuging, the organic phase was collected. Deionized water was used to clean 1 mL of the organic phase, resulting in the test solution. Refer to the next page for the analytical conditions for the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods. Analysis methods included in the GC/MS Metabolite Database Ver. 2 were used for the analysis conditions and monitoring *m/z*.

■Analysis Results

The sample extracted from the saury was measured in each analysis mode, and the separation from impurities investigated. Most of the fatty acid methyl esters could be separated from the impurities regardless of the analysis mode. However, a portion of the fatty acids was hard to completely separate from the impurities, both with EI-SIM and EI-MRM. Fig. 1.1.2 shows examples of measuring fatty acid methyl esters for which the degree of separation from impurities varied significantly depending on the analysis mode. Methyl linolenate;(Z)18:3n-3 and methyl *cis*-11,14,17-Icosatrienoate;(Z)20:3n-3 were hard to separate from the impurities, both with EI-SIM and EI-MRM. Some degree of separation was possible with PCI-SIM, but there was only one kind of monitoring *m/z*, so problems with peak identification could be expected. In contrast, with PCI-MRM, mass separation excluded impurities eluted nearby, making peak identification easy.

1.1 Analysis of Fatty Acids in Fish (2) - GC/MS/MS

■Analytical Conditions

Instrument	: GCMS-TQ8030
Column	: SP-2560 (Length 100 m; 0.20 mm I.D.; df = 0.25 µm)
Glass Insert	: Splitless insert with wool (P/N: 221-48876-03)
[GC]	
Injection Temp.	: 250 °C
Column Temp.	: 40 °C (2 min) - (4 °C /min) - 240 °C (15 min)
Injection Mode	: Split
Split Ratio	: 1:10
Carrier Gas Control	: Linear velocity (20.0 cm/sec)
Injection Volume	: 1 µL

[MS]	
Interface Temp.	: 250 °C
Ion Source Temp.	: 200 °C
Measurement Mode	:
GC/MS	: SIM
GC/MS/MS	: MRM
Ionization Method	: EI and PCI methods
PCI Reagent Gas	: Isobutane
PCI Reagent Gas Pressure	: 70 kPa

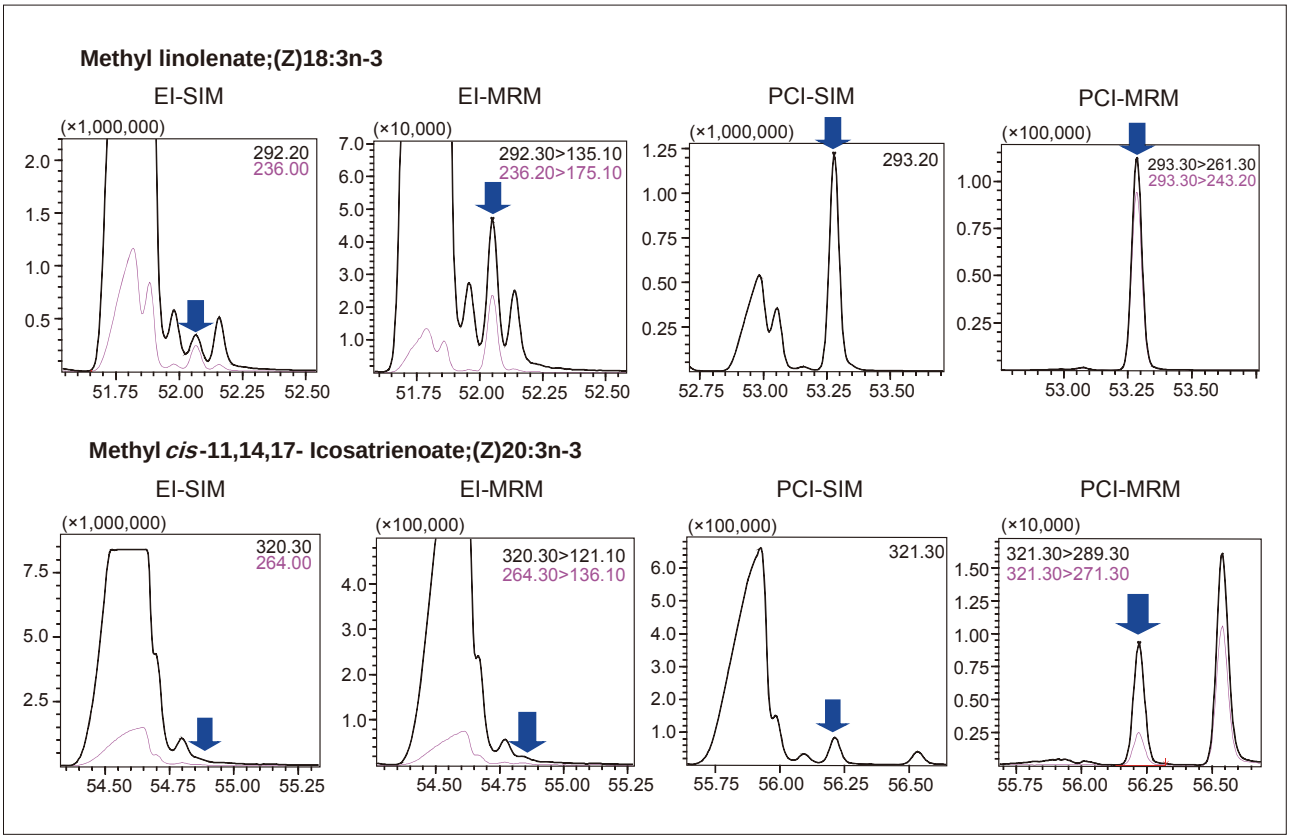


Fig. 1.1.2 Mass Chromatograms for Methyl linolenate;(Z)18:3n-3 and Methyl *cis*-11,14,17-Icosatrienoate;(Z)20:3n-3 Contained in an Extract of Saury Measured in Individual Analysis Modes

We analyzed fatty acids in fish to investigate mass separation from impurities and sensitivity for the EI-SIM, EI-MRM, PCI-SIM, and PCI-MRM analysis modes. The results revealed that the PCI method is the most sensitive, and for unsaturated fatty acids in particular, provides more sensitive detection than the EI method. Also, PCI-MRM was found to be the most ideal for mass separation from impurities, making peak identification easy. It is thus evident that the PCI-MRM method is effective for multicomponent batch analyses of fatty acids.

1.2 Analysis of Fatty Acids in Butter Using GC × GC/MS - GC/MS

■Explanation

Fig. 1.2.1 shows the results from GC × GC-MS analysis of methyl esterified lipids extracted from commercial butter by the Folch method. It confirms the main components, palmitic acid (C16) and oleic acid (C18 1ω6), as big blobs (see Fig 1.2.1). C18 fatty acids include a variety of components and isomers, but these can be separated into their respective components for highly accurate qualitative and quantitative results by using a second column with high polarity.

■Analytical Conditions

GC × GC Modulator : ZX1-GC × GC Modulator
GC-MS : GCMS-QP2010 Ultra

[GC × GC]
Column : 1st DB-1 (30 mL. × 0.25 mm I.D., 0.25 μm)
2nd Rtx-WAX (2.5 mL. × 0.1 mm I.D., 0.1 μm)
Injection Volume : 1.0 μL
Injection Mode : Split (split ratio 1:100)
Injection Temp. : 250 °C
Column Temp. : 40 °C (2 min) - (30 °C/min) - 160 °C (2 °C/min) -300 °C (5 min)
Control Mode : Constant pressure (150 kPa)
Modulation Time : 8 sec
Hot Pulse Time : 0.5 sec (325 °C)

[MS]
Interface Temp. : 240 °C
Ion Source Temp. : 200 °C
Solvent Elution Time : 15.5 min
Data Sampling Time : 16 min to 80 min
Measurement Mode : Scan
Mass Range : *m/z* 45-330
Event Time : 0.02 sec

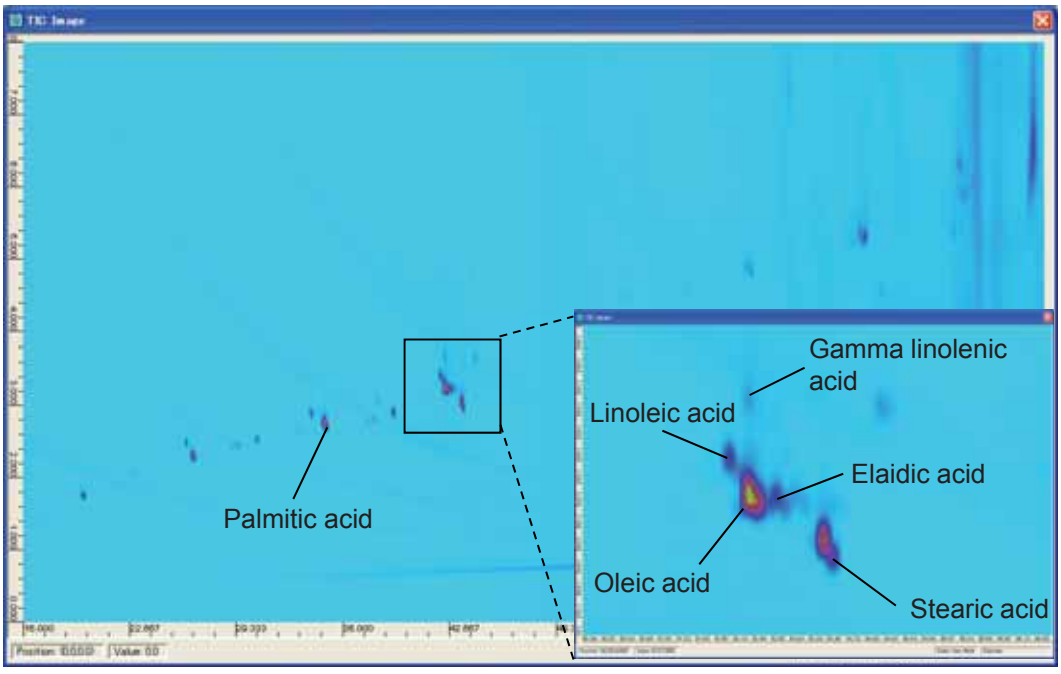


Fig. 1.2.1 2-Dimensional Image of GC × GC-MS Analysis Results for Butter

1.3 Quantitative Analysis of *Trans* Fatty Acid by FTIR (1) - FTIR

■Explanation

Trans fatty acids are a kind of fat that is found in foods. Excessive ingestion of this type of fat is associated with elevated LDL cholesterol (low density lipoprotein cholesterol) levels, which increases the risk of heart disease[1]. In Japan, there is currently, no obligation to display *trans* fatty acid content on food labels, nor is there a specified content limit in Japan. However, food entrepreneurs have been taking measures for some time by independently developing low *trans* fatty acid foods and selling them on the market. On the other hand, nations such as the United States and Denmark are obligated to display the *trans* fatty acid content on food labels and to adhere to content restrictions on *trans* fatty acids. Here we introduce a quantitative analysis study we conducted for *trans* fatty acids by the ATR and transmission methods.

■Measurement of *Trans* Fatty Acid by Single Reflection ATR Spectroscopy

According to the AOCS method, analysis of *trans* fatty acid content by FTIR is to be conducted by the transmission method[2] and the ATR[3] method. Here we used the latter method, which features easier operation. Fig. 1.3.1 shows the MIRacle A (ZnSe prism) single reflectance ATR (Attenuated Total Reflectance) accessory that was used to conduct measurement of the *trans* fatty acid content. A temperature controller was used during measurement to maintain the prism temperature at 65 °C (±2 °C). Unsaturated fatty acids are characterized as being either a *cis* type or *trans* type of isomer, as shown in Fig. 1.3.2. The samples used for analysis consisted of triolein (Glyceryl Trioleate, Sigma Corp.), with a single *cis* double bond, and trielaidin (Tokyo Chemical Industry, Co., Ltd.), having a *trans* type double bond. Table 1.3.1 shows the analytical conditions used, and Fig. 1.3.3 shows the infrared spectra of triolein and trielaidin. In the infrared spectrum of trielaidin, the characteristic *trans*-vinylene peak of the *trans*-type unsaturated fatty acid is seen at 966 cm^{-1}. Next, using triolein as a base, we prepared sample solutions spiked with trielaidin at concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 %, respectively, to generate a *trans* fatty acid calibration curve. The infrared spectra were measured similarly as described above. Fig. 1.3.4 shows the *trans* fatty acid peak in the vicinity of 966 cm^{-1} which was used to generate the calibration curve. An excellent correlation coefficient of 0.9999 was obtained with respect to the calibration curve generated from this, as shown in Fig. 1.3.5.

Fig. 1.3.1 PIKE MIRacle A ATR Accessory with Optional Heated Crystal Plate

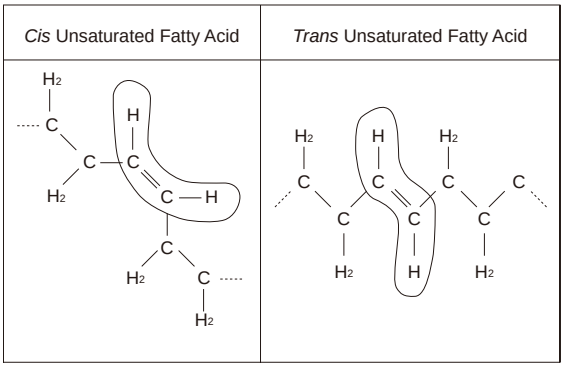


Fig. 1.3.2 Structures of *Cis* and *Trans* Fatty Acids

Table 1.3.1 Analytical Conditions

Analytical Instrument	: IRPrestige-21, MIRacle A (heating plate)
Resolution	: 4 cm^{-1}
Accumulation	: 45
Detector	: DLATGS

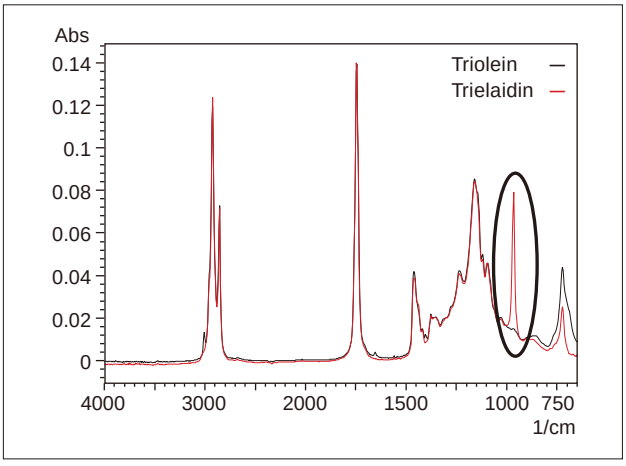


Fig. 1.3.3 Infrared Spectra of Triolein and Trielaidin

1.3 Quantitative Analysis of *Trans* Fatty Acid by FTIR (2) - FTIR

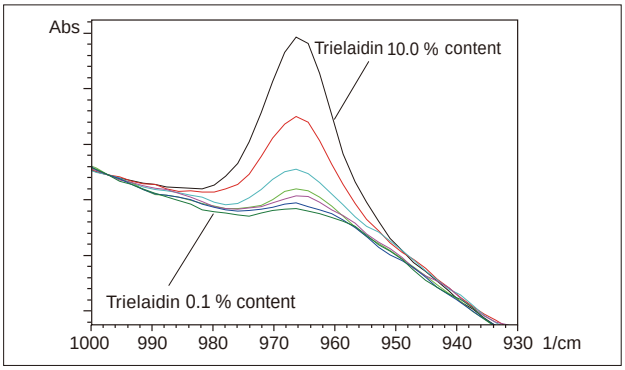


Fig. 1.3.4 *Trans* Fatty Acid Peak Region Selected for Calibration Curve

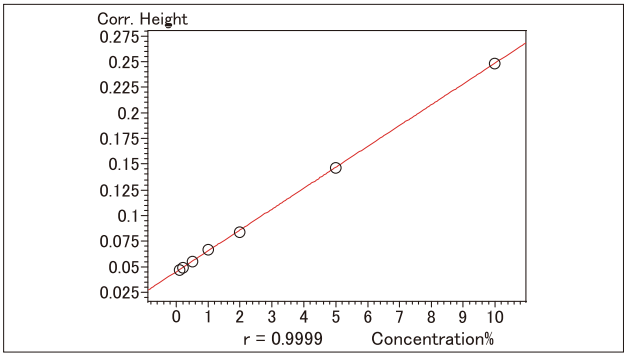


Fig. 1.3.5 Calibration Curve of *Trans* Fatty Acid by Single Reflection ATR Spectroscopy

Measurement of *Trans* Fatty Acid by Transmission Spectroscopy

We also conducted measurement by the transmission method. Detection with good S/N ratio is possible by selecting a fixed thickness cell with an appropriate wavelength. Here we used a 0.1 mm-fixed thickness cell (window plate material: KBr). This time, using as a base a commercially available olive oil in which the principle substance is a *cis* isomer, trielaidin-spiked samples were prepared at concentrations of 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 % respectively. Measurement was conducted by the ATR method using the same conditions as shown in Table 1.3.1.

The infrared spectra obtained from measurement of unspiked olive oil and 10.0 % trielaidin-spiked olive oil are shown in Fig. 1.3.6, Fig. 1.3.7 shows the *trans* fatty acid peak in the vicinity of 966 cm^{-1} which was used to generate the calibration curve. An excellent correlation coefficient of 0.9999 was obtained with respect to the calibration curve generated from this, as shown in Fig. 1.3.8.

[Reference]

1) Information on *Trans* Fatty Acids, Ministry of Agriculture, Forestry and Fisheries Web Page
http://www.maff.go.jp/j/syouan/seisaku/trans_fat/
2) AOCS Official Method Cd 14-95
Isolated *trans* Isomers infrared Spectrometric Method
3) AOCS Official Method Cd 14d-99
Rapid Determination of Isolated *trans* Geometric Isomers in Fats And oils by Attenuated Total Reflection Infrared Spectroscopy

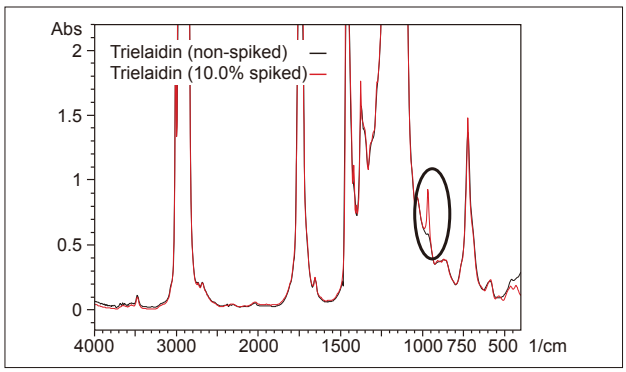


Fig. 1.3.6 Infrared Spectra of Unspiked Olive Oil and Olive Oil Spiked with Trielaidin

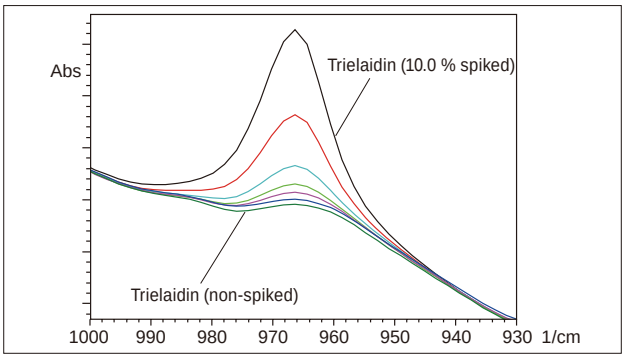


Fig. 1.3.7 *Trans* Fatty Acid Peak Region Selected for Calibration Curve

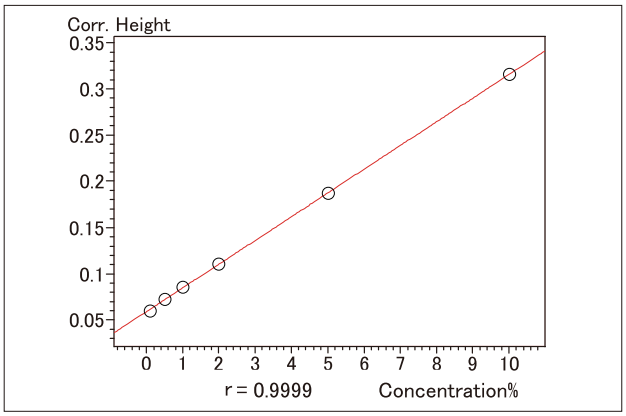


Fig. 1.3.8 Calibration Curve of *Trans* Fatty Acid by Transmission Spectroscopy

Conclusion

A calibration curve was generated using ATR method and transmission method for trielaidin-spiked samples prepared at concentrations from 0.1 % to 10.0 %. Using either method, an excellent correlation coefficient of 0.9999 was obtained, confirming that these methods are effective for quantitative analysis of *trans* fatty acids.

1.4 Analysis of 3-MCPD Fatty Acid Diesters in Palm Oil (1) - LC/MS/MS

■Explanation

3-MCPD (3-monochloropropane-1,2-diol) is a byproduct that is formed in the production of condiments such as soy sauce when hydrochloric acid is used to accelerate the hydrolysis of vegetable proteins such as defatted soybean and wheat gluten. According to the risk assessment of 3-MCPD by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 3-MCPD is not considered to be genotoxic or carcinogenic. However, animal tests have indicated that it adversely affects the kidneys if ingested in large quantities over a long period of time. In Japan, it has been confirmed that there is no 3-MCPD present in *honjozo* (authentically-brewed) soy sauce produced by a traditional method, which accounts for 85 % of the soy sauce produced in Japan. The general dietary intake of 3-MCPD that can be ingested without causing problems is not regulated in Japan. However, measures have been implemented to improve upon production methods and limit the inclusion of 3-MCPD. Recently, the presence of 3-MCPD fatty acid esters have been reported in many foods containing refined edible oils. The toxicity of 3-MCPD fatty acid esters has not yet been clarified, therefore the analysis of 3-MCPD fatty acid ester is very important. The application of GC/MS following derivatization with phenylboronic acid (DGF Standard methods 2009, Section C-Fats) has traditionally been used for analysis of 3-MCPD fatty acid esters, yet direct analysis by LC/MS/MS without derivatization is gaining attention as an attractive alternative method. Significant amounts of 3-MCPD fatty acid esters are present in numerous natural vegetable oils, and their concentration is particularly high in palm oil. Here, we introduce the quantitative analysis of 3-MCPD fatty acid esters in palm oil using LC/MS/MS.

■Analysis of Standard Samples

Synthetic 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester were used as standard samples. Electrospray ionization (ESI) was used as the ionization method and the 3-MCPD-di-fatty acid esters were detected as NH_4^+ adduct ions due to the addition of ammonium acetate in the mobile phase. The MS/MS spectra obtained using the adduct ions as the precursor are shown in Fig. 1.4.1. Varying the collision energies (CE) produced the MS/MS spectra in Fig. 1.4.1 with the top, middle, and bottom spectra generated by 10, 30, and 40 V, respectively. As each one of the fatty acids is desorbed, it is detected as a product ion. Fig. 1.4.2 shows the MRM chromatograms of the standard samples (1 µg/L).

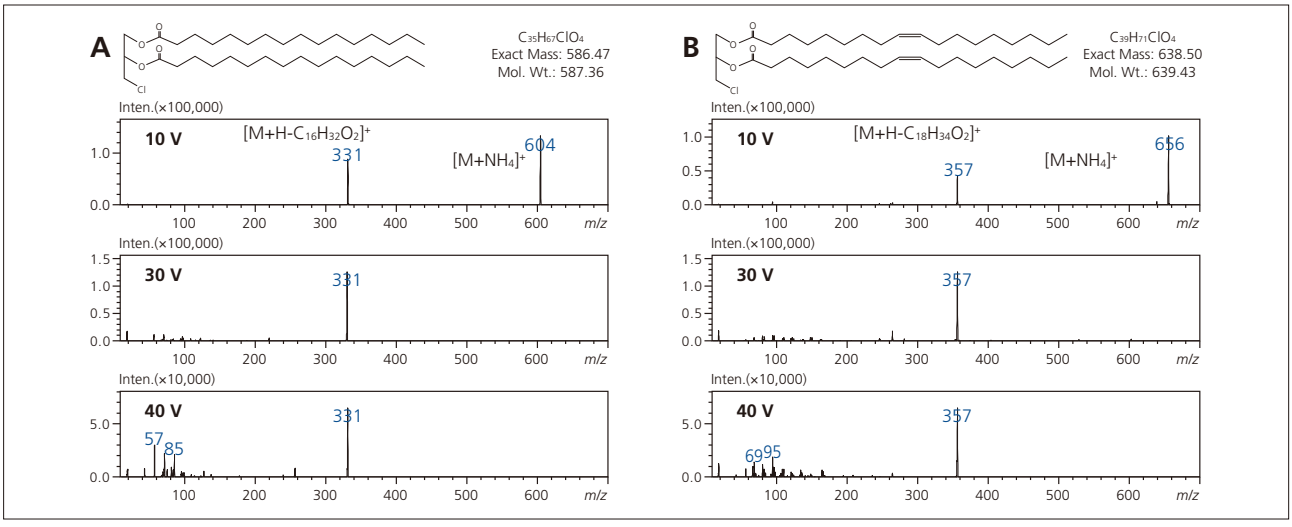


Fig. 1.4.1 MS/MS Spectra of the Synthetic Samples (A: 3-MCPD-Dipalmitoyl Ester, B: 3-MCPD-Dioleoyl Ester)

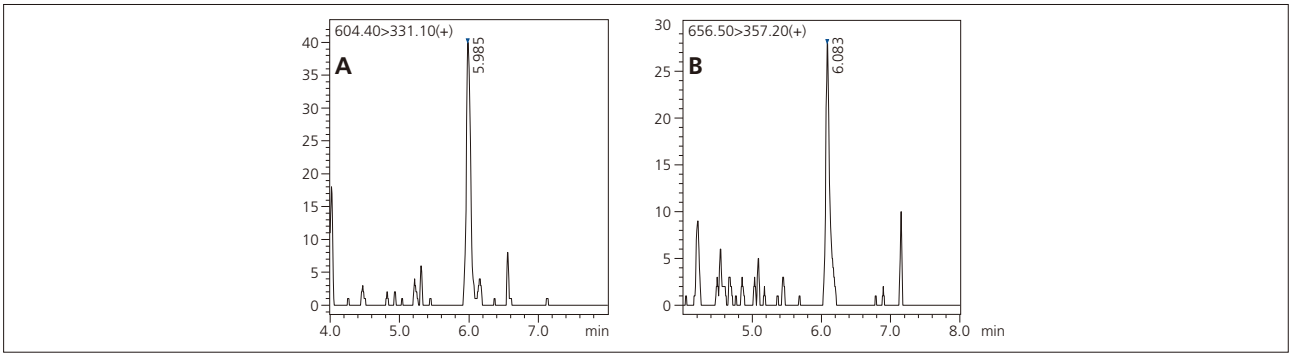


Fig. 1.4.2 MRM Chromatograms of the Synthetic Samples (1 µg/L, A: 3-MCPD-Dipalmitoyl Ester, B: 3-MCPD-Dioleoyl Ester)

1.4 Analysis of 3-MCPD Fatty Acid Diesters in Palm Oil (2) - LC/MS/MS

■Calibration Curve

The calibration curves for 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester are shown in Fig. 1.4.3A and Fig. 1.4.3B, respectively. Excellent linearity was obtained over a wide range from 1–1000 µg/L, with correlation coefficient (R^2) values greater than 0.999.

The repeatability using 6 repeat measurements of 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester was 15.47 and 19.64 area %RSD, respectively, at 1 µg/L, and 6.54 and 9.32, respectively, at 10µg/L.

■Analysis of Palm Oil

Fig. 1.4.4 shows an example of analysis of palm oil. 169.9 mg of palm oil was weighed out, dissolved in 1 mL of hexane and diluted 100 to 1 with acetone (588.6 times dilution), and then analyzed. 3-MCPD dipalmitoyl ester and 3-MCPD-dioleoyl ester were detected in this diluted solution at approximately 10 µg/L (Fig. 1.4.4A and Fig. 1.4.4B). This corresponds to a concentration in palm oil of about 6 mg/L of 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester, respectively. Thus, it is possible to use a triple quadrupole mass spectrometer for detection of 3-MCPD fatty acid esters using a simple pretreatment procedure that is limited to sample dilution.

■Analytical Conditions

Column	: Shim-pack XR-ODS Ⅲ (75 mm L. × 2.0 mm I.D., 2.2 µm)
Mobile Phase A	: Methanol with 3 mmol/L Ammonium Acetate / Acetonitrile = 9/1
Mobile Phase B	: Acetone / Methanol with 3 mmol/L Ammonium Acetate / Acetonitrile = 8/1/1 Gradient Elution Method
Time Program	: B 0 % (0-2.5 min) → 65 % (7.5 min) → 100 % (7.51-10 min) → 0 % (10.01-15 min)
Flowrate	: 0.4 mL/min
Column Temp.	: 40 °C
Injection Volume	: 2 µL
Probe Voltage	: 4.5 kV (ESI-Positive Mode)
Nenulizing Gas Flow	: 1.5 L/min
Drying Gas Flow	: 20 L/min
DL Temp.	: 300 °C
Block Heater Temp.	: 400°C
DL / Q-array Voltage	: Using default values

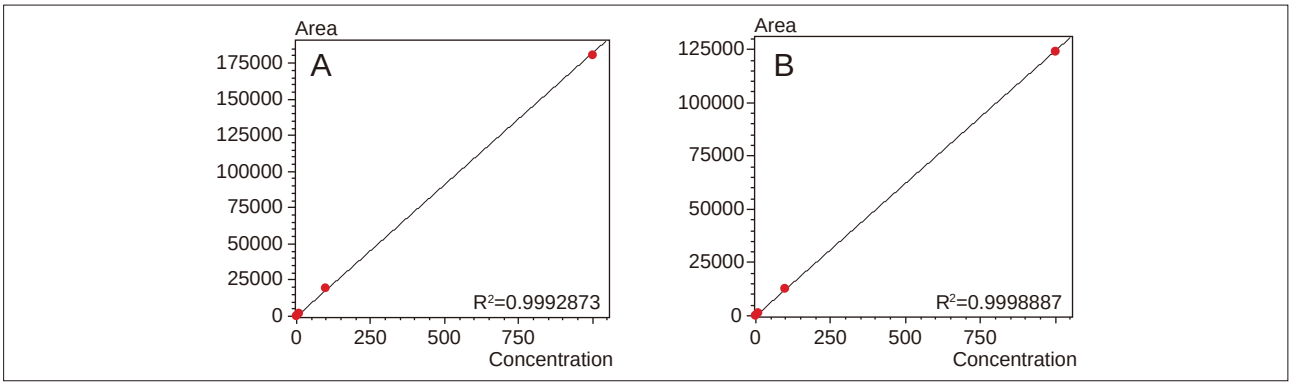


Fig. 1.4.3 Calibration Curves (1–1000 µg/L, n = 6)

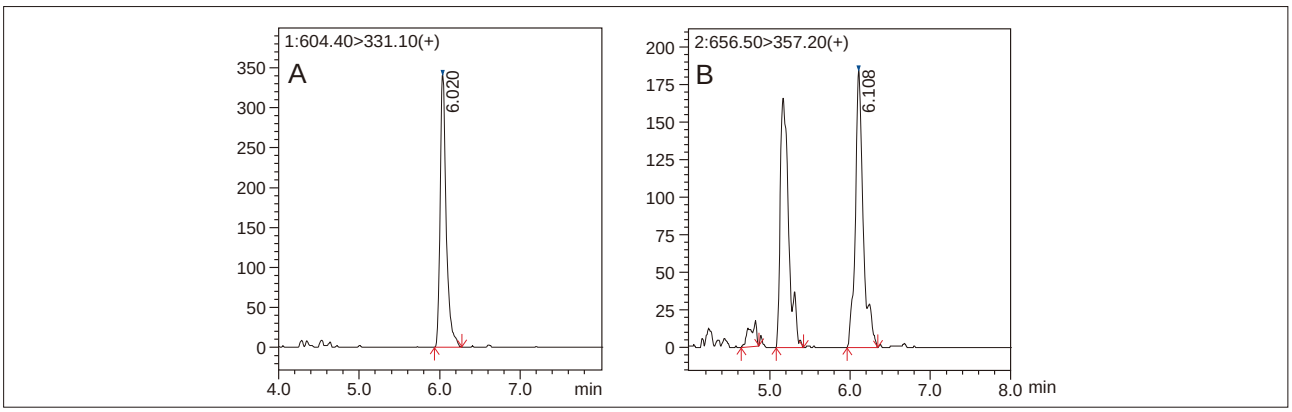


Fig. 1.4.4 MRM Chromatograms of 3-MCPD Fatty Acid Diesters in Palm Oil (A:3-MCPD-Dipalmitoyl Ester, B:3-MCPD-Dioleoyl Ester)

1.5 Quantitative Analysis of Fat in Milk by UV-Vis-NIR Reflectance Spectroscopy and Multivariate Analysis (1) - UV

■Explanation

Milk is one of the most popular drinks among humans. In recent years, however, there has been a great increase in the sales of milk products with adjusted fat content. Milk fat content is typically measured using the Roese-Gottlieb or the Gerber method, but measurement by these methods is extremely time-consuming. We therefore investigated the use of the spectral reflectance method as a simpler quantitative method. By applying a combination of reflectance measurement using a screw-top glass tube in conjunction with multivariate analysis, we found that the fat content could be determined quite easily. As multivariate analysis methods, the multiple linear regression method, PLS method, and support vector regression method (SVR) were used to conduct a comparative analysis of the quantitative accuracy of these quantitative methods. The results indicated that the support vector regression method provided the best quantitative accuracy. The results are introduced in this paper.

■Total Luminous Reflectance Measurement of Milk

Nine types of measurement samples with differing levels of fat content (3 types of high-fat milk, 3 types of medium-fat milk, 3 types of low-fat milk) were used. The fat content values displayed on the various milk cartons are shown in Table 1.5.1. To ensure that all of the samples were positioned identically, measurement was conducted with the integrating sphere mounted in a securing jig. Each sample was transferred to a screw-top glass tube which was then set in the integrating sphere as shown in Fig. 1.5.1, and the total light reflectance was measured twice for each sample by transferring the same sample to a different screw-top glass tube. Thus, a total of 18 data points were obtained (9 × 2 = 18). The disposable screw-top glass tubes were discarded after each use. In addition, a Spectralon® reflectance standard (U. S. Labsphere, Inc.) was used for the reflectance measurements.

The measurement results are shown in Fig. 1.5.2. In Fig. 1.5.2, the redcolored trace lines correspond to high-fat milk, the black trace lines to medium-fat milk, and the blue trace lines to low-fat milk. The data clearly indicate that the lower the fat content, the lower the reflectance becomes overall. An enlarged view of a portion of Fig. 1.5.2 is shown in Fig. 1.5.3. Looking at the blue-trace spectra of the low-fat milk, each of the two respective repeat measurement results overlap nearly perfectly, suggesting that exchanging the screw-top glass tube has little effect. The blue line spectra are clearly divided into 3 groups, corresponding to the descending order of fat content from 2.0, 1.0 and 0.2 (g/200 mL).

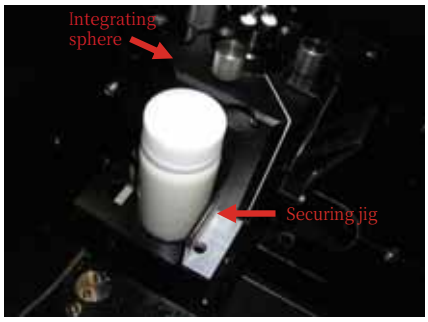


Fig. 1.5.1 Sample Set in Integrating Sphere

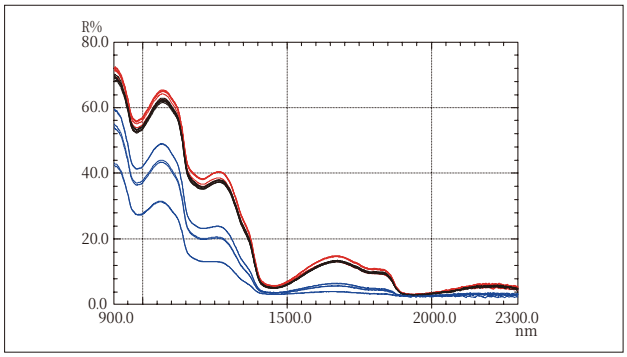


Fig. 1.5.2 Total Luminous Reflection Spectra of Samples
Red: High-Fat Milk, Black: Medium-Fat Milk, Blue: Low-Fat Milk

Table 1.5.1 Measurements of 9 Types of Milk Samples

Sample	Fat Content as Listed on Milk Carton (g/200 mL)
High-fat 1	9.3
High-fat 2	9.4
High-fat 3	9.5
Medium-fat 1	7.6
Medium-fat 2	7.8
Medium-fat 3	7.6
Low-fat 1	0.2
Low-fat 2	1.0
Low-fat 3	2.0

■Analytical Conditions

Instrument : UV-3600 UV-visible-near-infrared spectrophotometer
MPC-3100 Large sample compartment (with built-in integrating sphere)
Measurement Wavelength Range : 900 nm – 2300 nm
Scan Speed : Medium
Sampling Pitch : 1.0 nm
Photometric Value : Reflectance
Slit Width : (12) nm

1.5 Quantitative Analysis of Fat in Milk by UV-Vis-NIR Reflectance Spectroscopy and Multivariate Analysis (2) - UV

■Results of Quantitative Analysis

The multiple linear regression method of multivariate analysis, the PLS method, and support vector regression method (SVR) were applied to the acquired data to quantify the fat content. The first and second samples of the high-fat, medium-fat, and low-fat samples of Table 1.5.1 were used as standard samples to generate a calibration model. The fat content values displayed on the milk cartons were taken as the true fat content values of the standard samples. As for the support vector regression method, this can be considered as a type of quantitative method of kernel multivariate analysis which can also be applied to non-linear data. In addition, the number 3 samples of the respective fat content samples of Table 1.5.1 were used as verification samples to confirm the prediction accuracy of the calibration models. The fat content prediction results for the verification samples using each of the calibration models are shown in Table 1.5.2. The values in parentheses represent the error of the predicted value with respect to the fat content displayed on the milk carton. By comparing the amount of error among the various techniques, it was determined that the best results were obtained using the support vector regression method. It is thought that the relationship between the reflectance spectrum and concentration is not a linear relationship (proportional relationship). Compared to the multiple linear regression method and PLS method, which demonstrate their effectiveness with linear data, the support vector regression method may provide better results because it can also handle non-linear data effectively. Calculations associated with the PLS method and the support vector regression method were conducted using The Unscrambler® 1) multivariate analysis software of CAMO Software company. In addition, regression analysis calculations for the multiple linear regression method were conducted using the "Regression Analysis" feature the Microsoft Excel® 2) spreadsheet software.

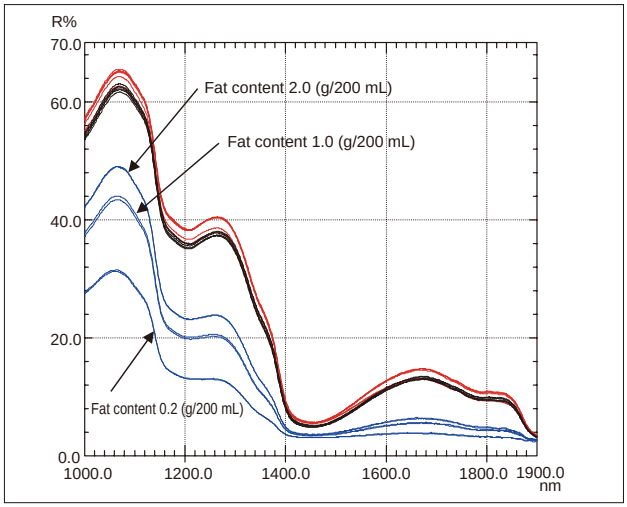


Fig. 1.5.3 Expanded Spectra of Fig. 1.5.2
Red: High-Fat Milk, Black: Medium-Fat Milk,
Blue: Low-Fat Milk

Note:

Calculations using the multiple linear regression method were conducted with respect to four wavelengths, 1100 nm, 1200 nm, 1500 nm and 1800 nm. As for the PLS method, all the data from 1100 to 1500 nm were used and mean centering was conducted to conduct the calculations. As for the support vector regression method, all the data from 1100 nm to 1500 nm were used, and the parameters C = 1 and ε = 0.08 were calculated using a linear kernel function.

1) The Unscrambler is a trademark or registered trademark of CAMO Software.
2) Excel is a trademark or registered trademark of Microsoft Corporation.

Table 1.5.2 Concentration of Fat for Samples and Results Calculated by Multiple Linear Regression, PLS Regression and Support Vector Regression

Sample	Fat Content Listed on Milk Carton (g/200 mL)	Predicted Results by Multiple Linear Regression Method	Predicted Results by PLS Method	Predicted Results by Support Vector Regression Method
High fat 3 (1st)	9.5	8.87 (6.6 %)	9.57 (0.7%)	9.72 (2.3 %)
High fat 3 (2nd)	9.5	8.89 (6.4 %)	9.67 (1.6 %)	9.47 (0.3 %)
Medium fat 3 (1st)	7.6	8.04 (5.8 %)	8.40 (10.5 %)	7.88 (3.7 %)
Medium fat 3 (2nd)	7.6	7.59 (0.1 %)	7.71 (1.4 %)	7.31 (3.8 %)
Low fat 3 (1st)	2.0	2.24 (12.0 %)	1.78 (11.0 %)	2.01 (0.5 %)
Low fat 3 (2nd)	2.0	2.36 (18.0 %)	1.72 (14.0 %)	1.86 (7.0 %)

■Conclusion

We conducted quantitation of fat content in various milk products by applying multivariate analysis to reflectance data. The result of a comparison of three types of multivariate analysis including the multiple linear regression method, the PLS method, and the support vector regression method indicated that the support vector regression method offered the best quantitative accuracy. In addition, while spectral transmission measurement of liquid samples typically involves time-consuming washing of the cell after each measurement, the current method which combines the use of the spectral reflectance method with disposable screw-top glass tubes eliminates the need for troublesome cleaning, while also saving time. The results obtained here suggest that this method is effective for the determination of fat content in highly turbid samples such as milk.

1.6 Analysis of Orotic Acid in Yogurt - LC

■Explanation

Orotic acid, a heteroaromatic compound discovered in whey, is also referred to as orotate, and uracil-6-carboxylic acid. In the past, it was also called vitamin B_{13}, but since it is synthesized in *vivo*, it is no longer considered to be a vitamin. Orotic acid is a major precursor of the nucleic acid pyrimidine, and has been attracting attention due to its diverse physiological effects. In Japan, it has been formulated into cosmetics and pharmaceuticals sold as "class 3 OTC drugs", namely OTC drugs with minimum risk of side effects. On January 23, 2012, the Pharmaceutical and Food Safety Bureau of the Japanese Ministry of Health, Labour and Welfare published a notification (No. 0123-3) regarding "a partial revision of the standards for a range of pharmaceutical products", stating that orotic acid, orotic acid potassium salt, and magnesium salt were added to the list of items specified as "ingredients that are not considered as drugs unless drug efficacy is advocated". As a result, its increased use in health foods, nutritional supplements, etc. can be expected. Here, we introduce an example of analysis of orotic acid in yogurt by reversed-phase chromatography using a Shimadzu Prominence HPLC system.

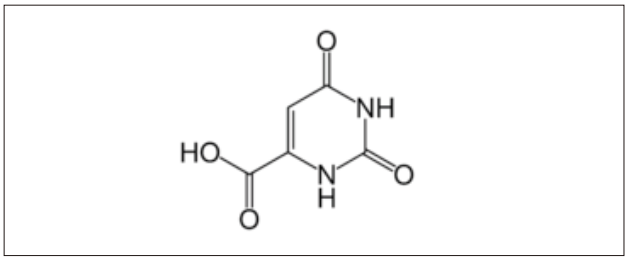


Fig. 1.6.1 Structure of Orotic Acid

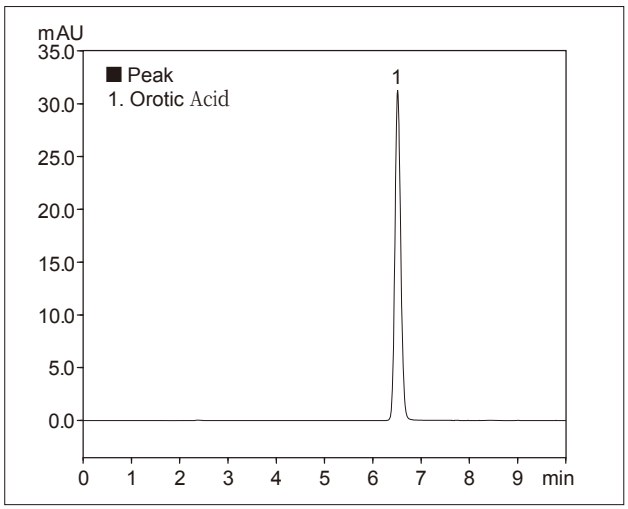


Fig. 1.6.2 Chromatogram of Standard Solution of Orotic Acid (10 mg/L, 10 µL injected)

■Analytical Conditions

Column	: Hydrosphere C18 (150 mm L. × 4.6 mm I.D., 5 µm, YMC Co., Ltd.)
Mobile Phase	: 10 mmol/L (Sodium) Phosphate Buffer (pH 2.6)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 10 µL
Detection	: SPD-20A at 280 nm

■Analysis of Yogurt

Fig. 1.6.3 shows a chromatogram of yogurt, and Fig. 1.6.4 shows that of a yogurt drink. After filtering the sample through an ultrafiltration membrane and then diluting it ten times with water, a 10 µL injection was made. The orotic acid content in the yogurt was found to be about 75 mg/L, and in the yogurt drink, about 35 mg/L.

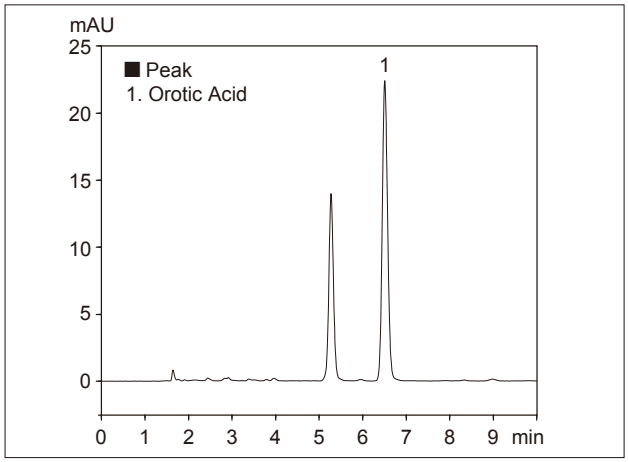


Fig. 1.6.3 Chromatogram of Yogurt (10 µL injected)

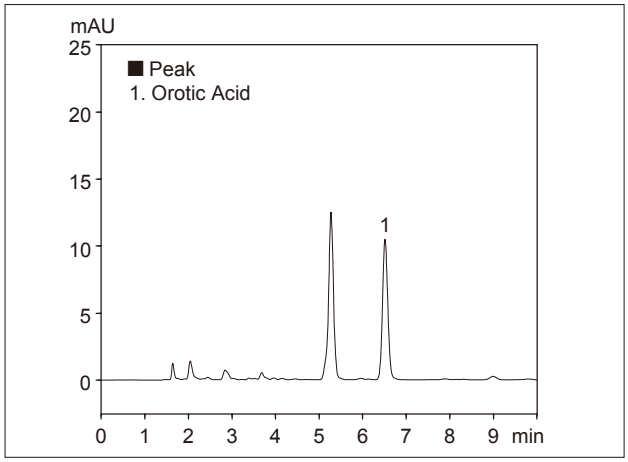


Fig. 1.6.4 Chromatogram of Yogurt Drink (10 µL injected)

1.7 High Speed Analysis of Organic Acids - LC

Explanation

HPLC analysis of organic acids (short-chain fatty acids) in food products is generally conducted using ion exclusion chromatography with post-column derivatization detection. However, this approach is limited from the standpoint of high-speed analysis. Here we show an example of high-speed, high-resolution analysis of organic acids with the "Prominence UFLC" ultra-fast HPLC system using reversed phase HPLC and an absorbance detector.

Analysis of a Standard Solution

Retention of organic acids like acetic acid and citric acid on a high-polarity, reversed phase column requires the use of 100 % aqueous mobile phases. However, such a mobile phase tends to adversely affect the longevity of silica type reversed phase columns. With that in mind, we conducted an investigation using the "Phenomenex Synergi Hydro- RP" ODS column (particle diameter 2.5 µm), in which the polar group is endcapped to not only strengthen the retention of polar compounds, but to better withstand 100 % aqueous mobile phases. Fig. 1.7.1 shows the chromatogram obtained from analysis of an organic acid standard mixture (formic acid, malonic acid, acetic acid, and citric acid, each 100 mg/L adjusted with mobile phase), using a 4 µL injection. Acidic phosphate buffer was used as the mobile phase, and ultraviolet detection was carried out at 210 nm. With these analytical conditions, the organic acids were separated in under 2 minutes.

Analytical Conditions

Column	: Phenomenex Synergi 2.5 µm Hydro-RP 100 Å (100 mm L. × 3.0 mm I.D., 2.5 µm)
Mobile Phase	: 10 mmol/L (Sodium) Phosphate Buffer (pH 2.6)
Flowrate	: 0.8 mL/min
Column Temp.	: 30 °C
Injection Volume	: 4 µL
Detection	: SPD-20A at 210 nm
Flow Cell	: Semi-micro cell

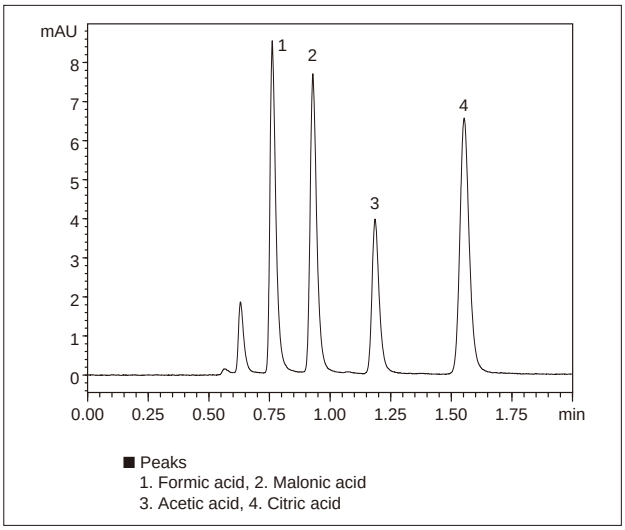


Fig. 1.7.1 Chromatogram of a Standard Mixture of 4 Organic Acids (100 mg/L each, 4 µL injected)

Analysis of Soft Drinks

Figs. 1.7.2 and 1.7.3 show chromatograms of commercially available soft drinks. After soft drink A and soft drink B were diluted fifty-fold and twenty-fold, respectively, with mobile phase, and then filtered through a membrane filter (pore diameter 0.2 µm), 4 µL of filtrate was injected for each analysis.

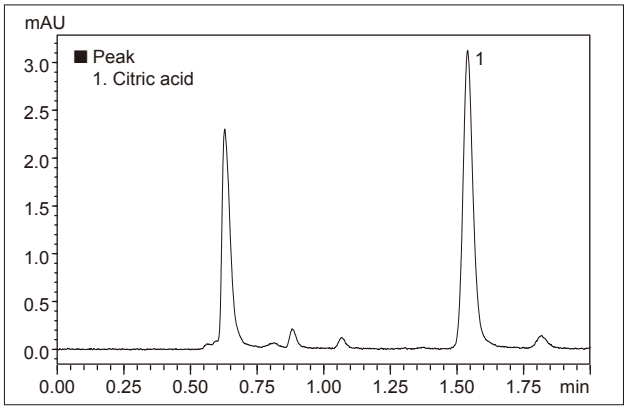


Fig. 1.7.2 Soft Drink A (50 × dilution, 4 µL injected)

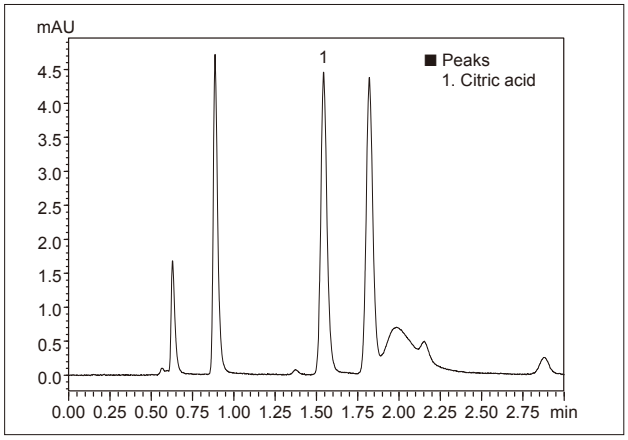


Fig. 1.7.3 Soft Drink B (20 × dilution, 4 µL injected)

*Cautions Regarding This Analysis

- Sample injection
  Since a buffered solution is used as the mobile phase, the sample solvent and injection volume may affect the results. For example, if a large volume of an alcoholic drink is injected, peak distortion may occur.
- Detection selectivity
  Since detection is conducted at UV 210 nm, the results are easily affected by impurities. Analysis may be difficult if large quantities of impurities are present in the sample.
- Column washing
  If 100 % buffer solution is used as the mobile phase, elution of the organic acids may gradually occur earlier. In this situation, conduct column washing with mobile phase containing some amount of an organic solvent (example: water/acetonitrile = 1/1). This washing is also effective for removing hydrophobic constituents from the sample.

1.8 Analysis of Amino Acids Contained in Vegetable Juice (1) - GC/MS

■Explanation

Amino acids contained in vegetable juice were treated with EZ: faast™ (Phenomenex, Inc.), which enables easy pretreatment, and then analyzed with a GC-MS system. Two kinds of vegetable juice were treated with EZ: faast. Norvaline was added as an internal standard. A GCMS-QP2010 Ultra (with high-power oven) was used for the measurements. The analysis conditions were in conformity with the "Amino Acid Analysis Methods" in the "GC/MS Metabolic Components Database."

■Analytical Conditions

Instrument : GCMS-QP2010 Ultra (with high-power oven)
Column : ZB-AAA (length: 10 m, 0.25 mm I.D.) (Phenomenex, Inc.)

[GC]		[MS]	
Injection Volume	: 1 µL	Interface Temp.	: 280 °C
Injection Temp.	: 280 °C	Ion Source Temp.	: 200 °C
Column Temp.	: 110 °C - (30 °C/min) -320 °C	Solvent Elution Time	: 0.4 min
Control Mode	: Constant pressure (15 kPa)	Data Sampling Time	: 0.5 min to 7 min
Injection Mode	: Split	Measurement Mode	: Scan
Split Ratio	: 1:15	Mass Range	: *m/z* 45-450 (3,333 u/sec)
Carrier Gas	: He	Event Time	: 0.15 sec

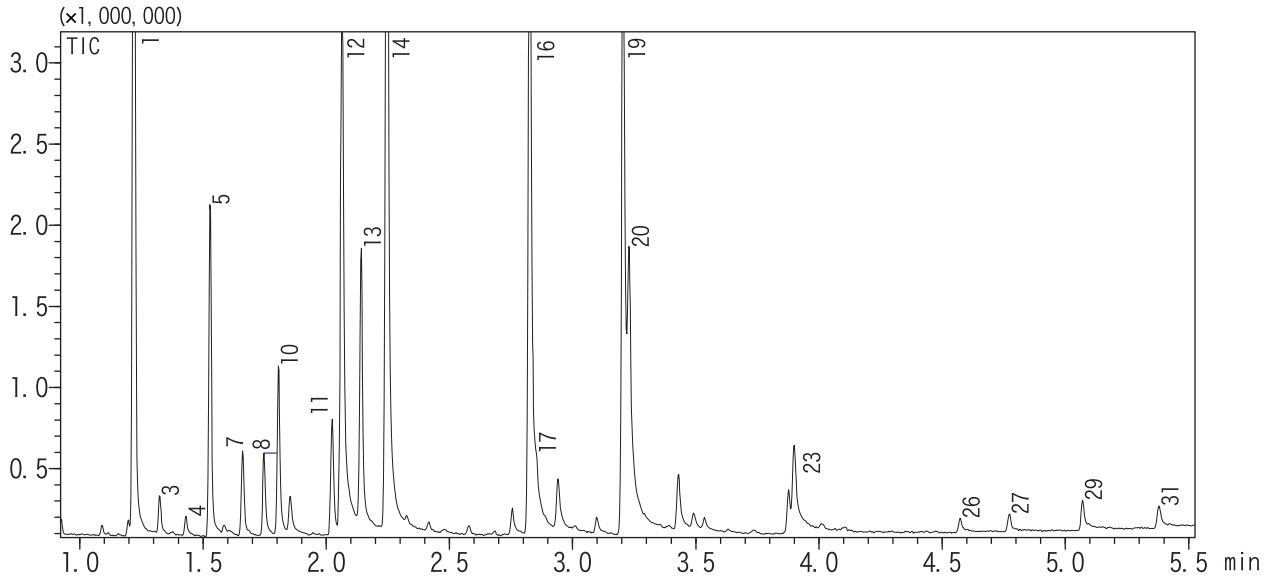


Fig. 1.8.1 Total Ion Current Chromatogram (TIC) for Amino Acid Derivatives in Vegetable Juice A

The numbers for each component follow the serial numbers in the "GC/MS Metabolic Components Database."

1 Alanine	8 Leucine	14 Asparagine	23 Glutamine
3 Glycine	10 Isoleucine	16 Aspartic acid	26 Lysine
4 alpha-aminobutyric acid	11 Threonine	17 Methionine	27 Histidine
5 Valine	12 Serine	19 Glutamic acid	29 Tyrosine
7 Norvaline(I.S.)	13 Proline	20 Phenylalanine	31 Tryptophan

1.8 Analysis of Amino Acids Contained in Vegetable Juice (2) - GC/MS

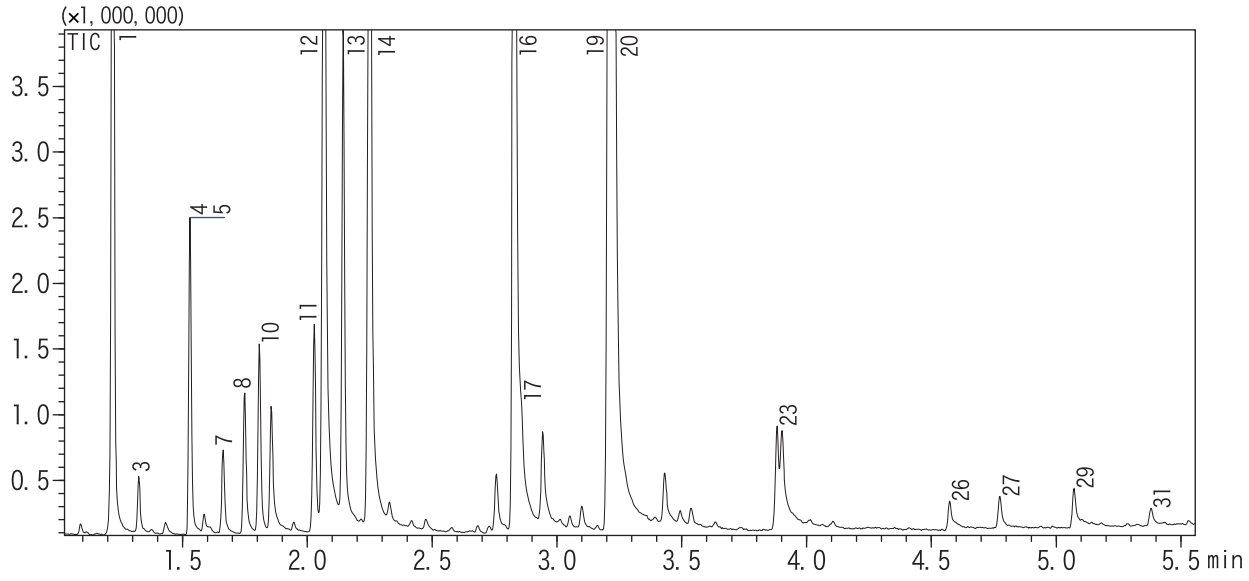


Fig. 1.8.2 Total Ion Current Chromatogram (TIC) for Amino Acid Derivatives in Vegetable Juice B

The numbers for each component follow the serial numbers in the "GC/MS Metabolic Components Database."

1 Alanine	8 Leucine	14 Asparagine	23 Glutamine
3 Glycine	10 Isoleucine	16 Aspartic acid	26 Lysine
4 alpha-aminobutyric acid	11 Threonine	17 Methionine	27 Histidine
5 Valine	12 Serine	19 Glutamic acid	29 Tyrosine
7 Norvaline (I.S.)	13 Proline	20 Phenylalanine	31 Tryptophan

■Summary

Pretreatment using the EZ: faast kit, following by analysis using the GCMS-QP2010 Ultra, which is equipped with a high-speed scanning function, enabled rapid analysis of amino acids. With this combination, it took only 15 minutes per sample from pretreatment to analysis.

1.9 High Speed Analysis of Pre-Column Derivatized Amino Acids by SIL-30AC Auto-sampler (1) - LC

■Explanation

Amino acid analysis is required in a wide range of fields, including foods and pharmaceuticals, and various methods of derivatization have been devised to improve sensitivity and selectivity when conducting amino acid analysis by HPLC. Here, using the RF-20Axs fluorescence detector and the SIL-30AC autosampler with its automated pretreatment functions, we introduce the analysis of amino acids using pre-column derivatization with OPA and FMOC.

■Simultaneous Determination of 22 Amino Acids

Using the automated pretreatment functions of the Nexera SIL-30AC autosampler, primary and secondary amino acids were automatically derivatized into fluorescent substances within the autosampler using *o*-phthalaldehyde (hereafter, OPA) and 9-fluorenyl methyl chloro formate (hereafter, FMOC), respectively. After separation of the derivatized amino acids using the ultra-high speed YMC-Triart C18 column (1.9 µm, YMC Co., Ltd.), high-sensitivity detection was conducted using the RF-20Axs fluorescence detector. Since the OPA-derivatized amino acids and FMOC-derivatized amino acids are detected at different wavelengths, simultaneous analysis was conducted utilizing the automatic wavelength switching feature. Table 1.9.1 shows the derivatization reagents used in this method, and Fig. 1.9.1 shows the reagent addition and mixing steps used to conduct the automated derivatization using the SIL-30AC autosampler. Since a constant reaction time can be maintained with the automated derivatization using the autosampler, excellent repeatability can be obtained compared with pre-column derivatization by manual operation. Fig. 1.9.2 shows the chromatogram.

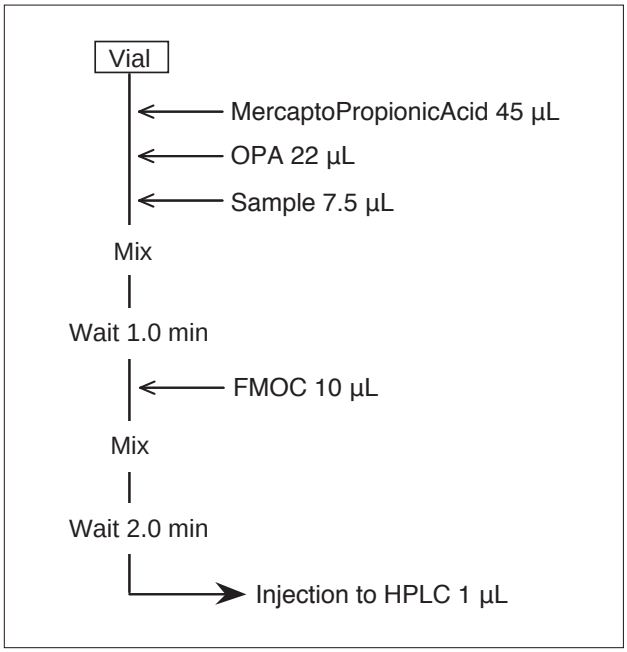


Fig. 1.9.1 Flowchart of Automated Pre-Column Derivatization with SIL-30AC

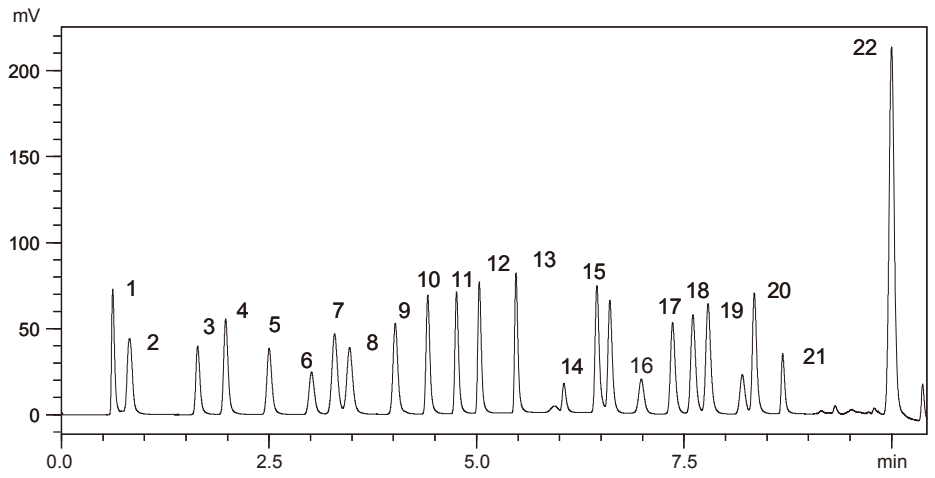
Table 1.9.1 Derivatization Reagents

- **Mercaptopropionic Acid**
  3-Mercaptopropionic Acid 10 µL in 0.1 mol/L Borate Buffer (pH 9.2) 10 mL
- ***o*-Phthalaldehyde Solution**
  o-Phthalaldehyde 10 mg in 0.1 mol/L Borate Buffer (pH 9.2) 5 mL
- **Fluorenyl Methyl Chloro Formate - Acetonitrile Solution**
  9-Fluorenyl Methyl Chloro Formate 4 mg in Acetonitrile 20 mL

■Analytical Conditions

Column : YMC-Triart C18, 1.9 µm
(75 mm L. x 3.0 mm I.D., 1.9 µm, YMC Co., Ltd.)
Mobile Phase : A: 20 mmol/L (Potussium) Phosphate Buffer (pH 6.9)
B : 45/40/15 Acetonitrile / Methanol / Water
Gradient Elution Method
Time Program : B 11 % → 13 % (0.00-3.00 min)
→ 31 % (5.00 min) → 37 % (7.5 min)
→ 70 % (10.00 min) → 100 % (10.50-13.50 min)
→ 11 % (14.00 min)
Flowrate : 0.8 mL/min
Column Temp. : 35 °C
Injection Volume : 1 µL
Detection : RF-20Axs Ex. at 350 nm, Em. at 450 nm
→ Ex. at 266 nm, Em. at 305 nm (9.0 min)
Cell Temp. : 20 °C
Flow Cell : Conventional cell

1.9 High Speed Analysis of Pre-Column Derivatized Amino Acids by SIL-30AC Auto-sampler (2) - LC



■Peaks
1. Aspartic Acid 2. Glutamic Acid 3. Asparagine 4. Serine 5. Glutamine 6. Histidine 7. Glycine 8. Threonine 9. Citruline
10. Arginine 11. Alanine 12. GABA 13. Tyrosine 14. Cys-Cys 15. Valine 16. Methionine 17. Tryptophan 18. Phenylalanine
19. Isoleucine 20. Leucine 21. Lysine 22. Proline

Fig. 1.9.2 Chromatogram of 22 Amino Acids (10 µmol/L Each, 1 µL Injection)

■Linearity and Repeatability

Using calibration curves generated with a concentration range from 1-100 µmol/L for each amino acid, excellent linearity was obtained, with a ratio of contribution (R^2 value) greater than 0.999 in all cases. Table 1.9.2 shows the peak area repeatability for all 22 amino acids obtained in repeat analysis (n = 6).

Table 1.9.2 Repeatability

	Area%RSD		Area%RSD
Asp	0.50	GABA	0.41
Glu	0.48	Tyr	0.55
Asn	0.51	Cys-Cys	0.46
Ser	0.41	Val	0.71
Gln	0.56	Met	0.71
His	0.57	Trp	0.70
Gly	0.29	Phe	0.73
Thr	0.55	Ile	0.63
Cltruline	0.46	Leu	0.55
Arg	0.45	Lys	0.56
Ala	0.46	Pro	2.35

■Analysis of Actual Samples

Fig. 1.9.3 shows a chromatogram of analysis of a commercially available soft drink using this method. The sample was analyzed after filtering it through a 0.2 µm membrane filter.

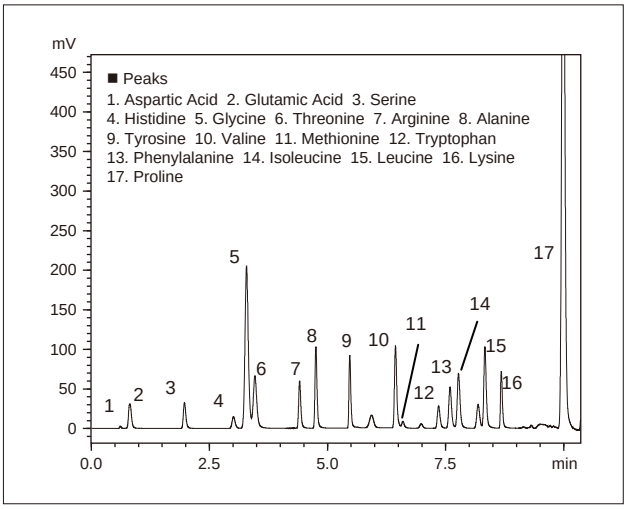


Fig. 1.9.3 Chromatogram of Soft Drink

1.10 High Speed Analysis of Pre-Column Derivatized Amino Acids in Alcoholic Beverage (1) - LC

■Explanation

In the previous section we introduced the analysis of amino acids that are obtained by hydrolysis primarily of proteins. Amino acids were prepared by automated precolumn derivatization using the SIL-30AC.

However, for amino acid analysis applications which require the search for functional constituents, etc. in foods, monitoring of even more types of amino acids is becoming necessary. Here, we introduce an example of the determination of 26 amino acids using a different column size and different mobile phase conditions than were used in the previous section. The automatic pretreatment feature of the SIL-30AC was utilized for derivatization of the amino acids during analysis, thereby enabling the overall analysis time to be substantially shortened.

■Simultaneous Determination of 26 Amino Acids

The automatic pretreatment features of the Nexera SIL-30AC autosampler were utilized to conduct automated derivatization of primary amino acids using *o*-phthalaldehyde (hereafter, OPA) and secondary amino acids, such as proline, etc., using 9-fluorenyl methyl chloro formate (hereaf ter, FMOC), to produce fluorescent substances within the autosampler. For the derivatization reagents used in this method and the pretreatment program for the SIL-30AC, refer to the previous section. Fig. 1.10.1 shows the chromatogram obtained from analysis of a standard mixture of 26 amino acids in solution.

■Analytical Conditions

Column : YMC-Triart C18, 1.9 µm
(100 mm L. × 2.0 mm I.D., 1.9 µm, manufactured by YMC Co., Ltd.)

Mobile Phase : A : 20 mmol/L (Potussium) Phosphate Buffer (pH 6.5)
B : 45/40/15 Acetonitrile/Methanol/Water
Gradient Elution Method

Time Program : B 11 % (0.00 - 2.00 min) → 17 % (4.00 min) → 31 % (5.50 min) → 32.5 % (10.00 min) → 46.5 % (12.00 min) → 55 % (15.50 min) → 100 % (15.51 - 19.00 min) → 11 % (19.01 min)

Flowrate : 0.4 mL/min

Column Temp. : 35 °C

Injection Volume : 1 µL

Detection : RF-20Axs, Ex. at 350 nm, Em. at 450 nm → Ex. at 266 nm, Em. at 305 nm (14.0 min)

Cell Temp. : 25 °C

Flow Cell : Semi-micro cell

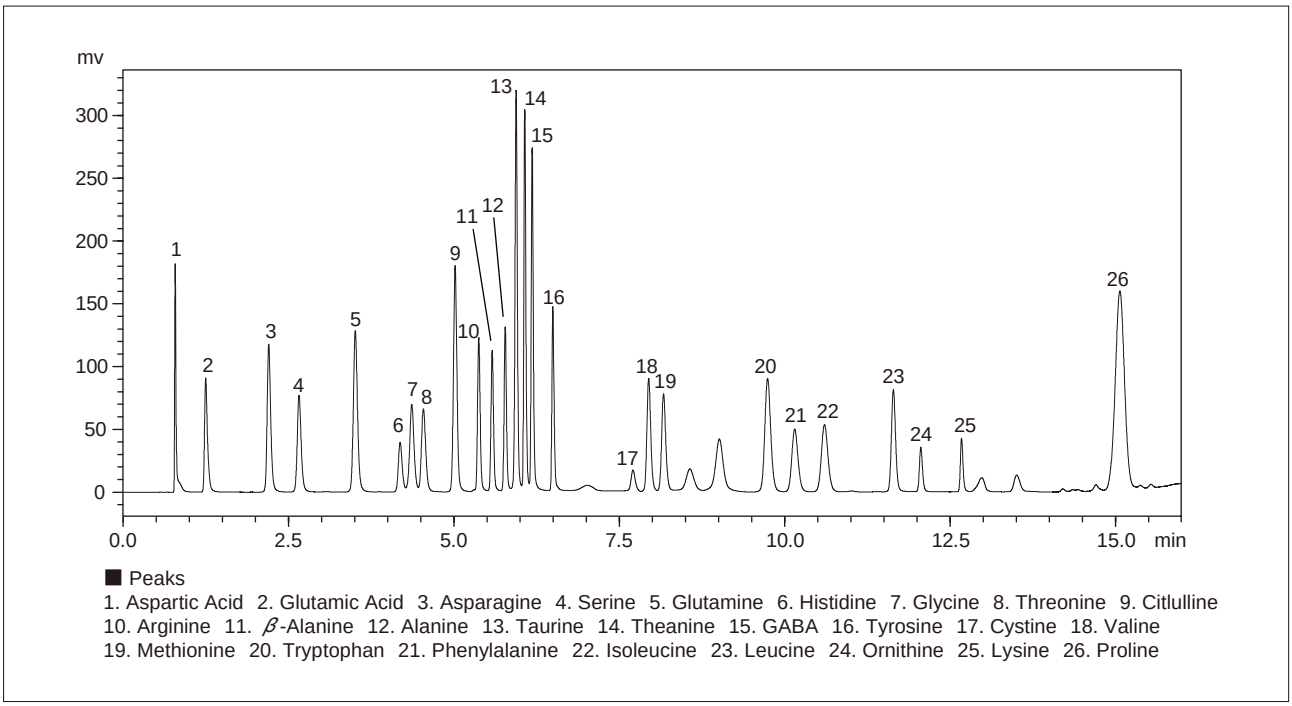


Fig. 1.10.1 Chromatogram of Standard Mixture Solution of 26 Amino Acids (10 µmol/L Each)

1.10 High Speed Analysis of Pre-Column Derivatized Amino Acids in Alcoholic Beverage (2) - LC

■Application of Overlapping Injection Pretreatment Feature

The overlapping injection function of the SIL-30AC allows preparation of the next sample to occur during the current analysis operation, thereby shortening the time necessary for a series of analyses. In the amino acid analysis presented here, the time required to complete an analysis cycle is shortened by conducting sample derivatization (reagent addition and mixing) of the subsequent sample during the current analysis. A derivatized sample is injected into the column, and when the analysis starts, the autosampler begins to prepare the next sample by adding reagent and mixing the contents. Fig. 1.10.2 shows a flow diagram description of the overlapping injection analysis cycle.

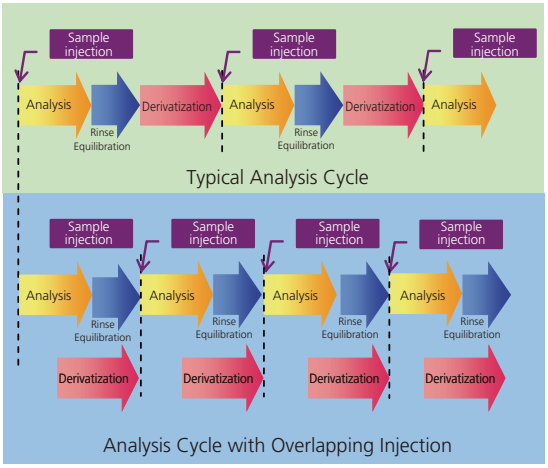


Fig. 1.10.2 Automated Derivatization Using Overlapping Injection

■Analysis of Alcoholic Beverages

Two commercially available alcoholic beverages were analyzed using the automated pre-column derivatization. Fig. 1.10.3 shows each of the chromatograms. After diluting each of the samples with 0.1 mol/L HCl, they were filtered through a 0.2 µm membrane filter, and then derivatized by the SIL-30AC pretreatment procedure. 1 µL of each was injected.

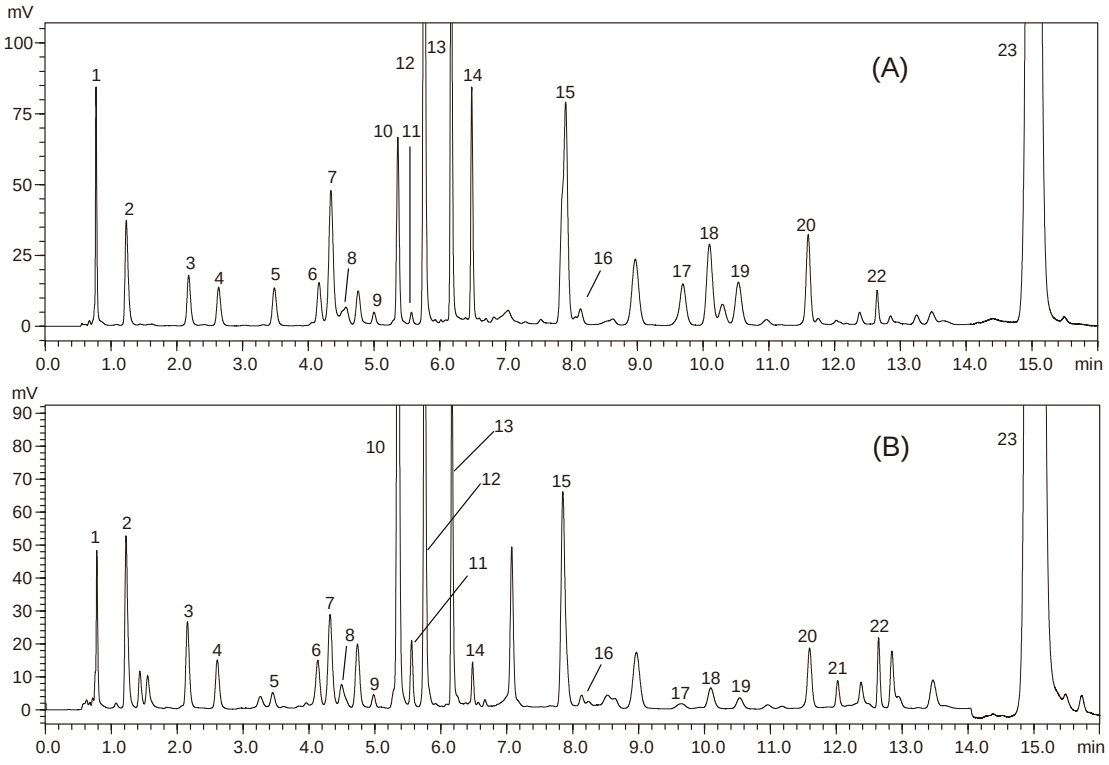


Fig. 1.10.3 Chromatograms of Alcoholic Beverages: (A) Beer (B) White Wine

1.11 Ultra High-Sensitivity Analysis of Water-Soluble Vitamins - LC

■Explanation

The Nexera SR is a high-end model within the Nexera X2 series of ultra high performance liquid chromatographs. It features the SPD-M30A high-sensitivity photodiode array detector which incorporates the newly designed capillary SR-Cell (Sensitivity and Resolution Cell). Optimization of the optical path length and diameter results in both high sensitivity and low noise. Introduced here is an example of high-speed, high-sensitivity simultaneous analysis of water-soluble vitamins using the Nexera SR ultra high performance liquid chromatograph with high-sensitivity cell (option).

■Simultaneous Analysis of 6 Water-Soluble Vitamins

High-sensitivity cell (option) of the Nexera SR ultra high performance liquid chromatograph incorporates 85 mm optical path length. Low noise levels and long optical path length have achieved excellent S/N, not only high signal response. In this simultaneous analysis of water-soluble vitamins, S/N has increased by 7.0 times compared to the previous instrument. High sensitivity detection is achieved even for compounds with low molar absorptivity.

■Analytical Conditions

Column	: Kinetex 2.6 µm C18 100 Å (100 mm L. × 4.6 mm I.D., 2.6 µm)
Mobile Phase	: A: 20 mmol/L (Sodium) Phosphate Buffer (pH2.5) 2 mmol/L Sodium 1-Hexanesulfonate B: Mobile PhaseA/Acetonirtile = 2/3 Gradient Elution Method
Time Program	: B 5 % (0.0 min.) → 23 % (1.0 min.) → 100 % (2.0-2.5 min.)
Flowrate	: 2.5 mL/min
Column Temp.	: 40 °C
Injection Volume	: 5 µL

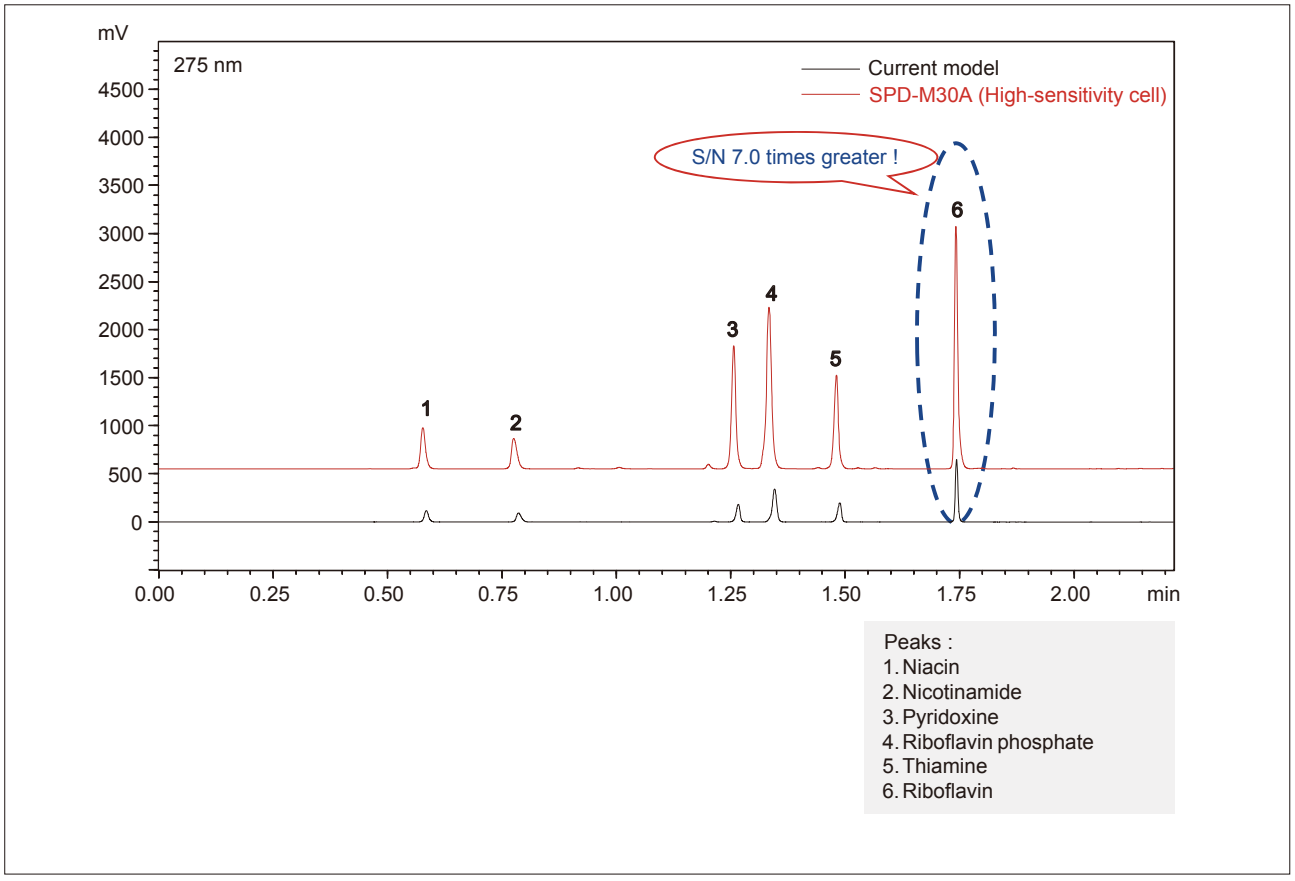


Fig. 1.11.1 Chromatogram of a Standard Mixture Solution of 6 Water-Soluble Vitamins

1.12 High-Sensitivity Analysis of Retinol Acetate and Retinol Palmitate - LC

■Explanation

In everyday use, the term "Vitamin A" is synonymous with retinol. Because retinol is a substance that is easily oxidized, highly stable derivatives of retinol are used in such products as foods, medicines and cosmetics. In HPLC analysis of stable retinol derivatives such as retinol acetate and retinol palmitate, a fluorescence detector offers both selectivity as well as highsensitivity analysis. Here we introduce examples of high-sensitivity analysis of retinol acetate and retinol palmitate, in addition to ultra-high speed analysis of retinol palmitate using the Prominence RF-20Axs fluorescence detector.

■Analysis of Standard Solution

Fig. 1.12.1 shows the structures of retinol, retinol acetate, and retinol palmitate. Fig. 1.12.2 shows an example of analysis of a retinol acetate and retinol palmitate standard solution (1.2 µg/L and 1.1 µg/L in methanol solvent). This confirmed that the Prominence RF-20Axs is capable of detecting these retinol derivatives at concentrations as low as 10 pg.

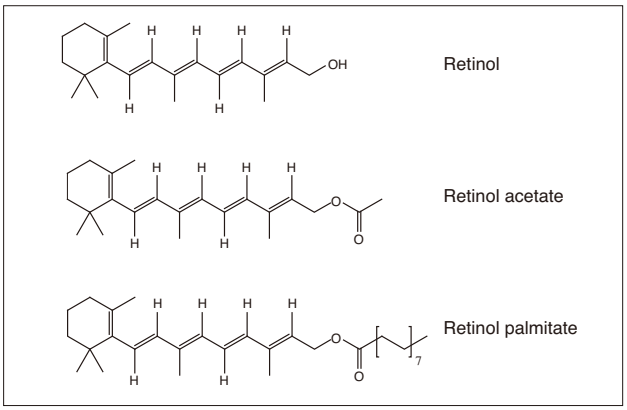


Fig. 1.12.1 Structures of Retinol, Retinol Acetate and Retinol Palmitate

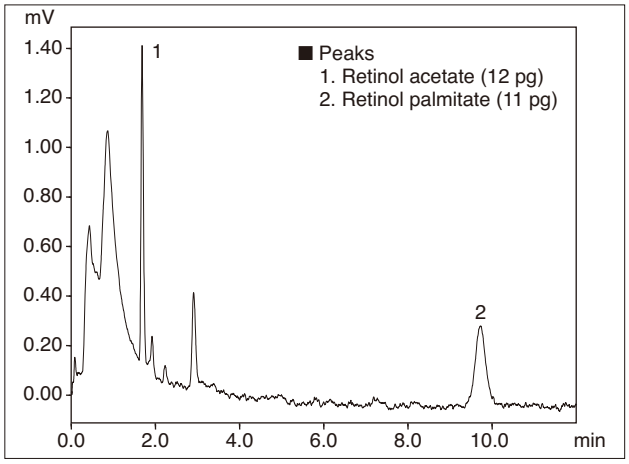


Fig. 1.12.2 Chromatogram of a Standard Mixture of Retinol Acetate (1.2 µg/L) and Retinol Palmitate (1.1 µg/L) (10 µL injected)

■Analytical Conditions

Column	: Shim-pack FC-ODS (75 mm L. × 4.6 mm I.D., 3 µm)
Mobile Phase	: Methanol
Flowrate	: 1.2 mL/min
Column Temp.	: 35 °C
Injection Volume	: 10 µL
Detection	: RF-20Axs Ex. at 350 nm, Em. at 480 nm
Cell Temp.	: 25 °C
Flow Cell	: Conventional cell

■Analysis of Multivitamin Tablets

Fig. 1.12.3 shows an example of analysis of the multivitamin tablets. The multivitamin tablets were crushed, extracted in methanol (ultrasonic extraction), centrifuged, and then diluted in methanol. After filtering through a membrane filter, 10 µL was injected.

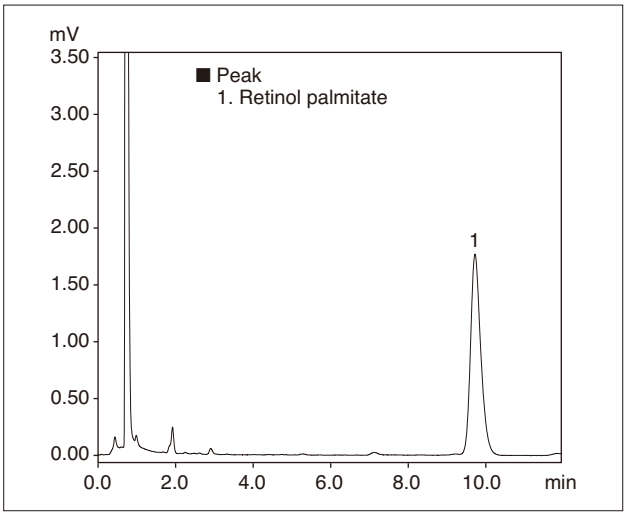


Fig. 1.12.3 Chromatogram of Multivitamin Tablets (10 µL injected)

1.13 Analysis of Water-Soluble Vitamins with Multi-Mode ODS Column (1) - LC/MS

■Explanation

Dietary guidelines and nutritional supplements are a significant concern to health conscious consumers. Upper and lower limits of daily intake of such functional foods have been specified for 12 vitamins and 2 minerals. Water-soluble vitamins are one class of nutrients whose measurement is important to the food and nutritional supplement industries. Due to their high polarity, their retention is extremely weak when using reversed-phase chromatography. Historic use of ion-pair reagents when conducting LC/MS analysis of such weakly retained analytes has resulted in reduced sensitivity.

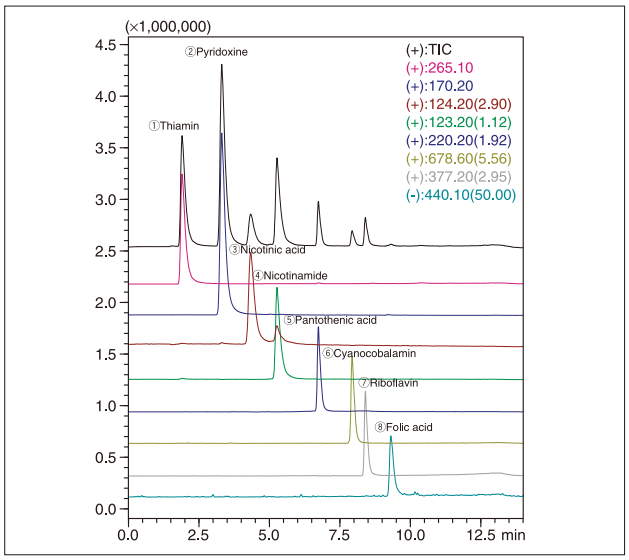


Fig. 1.13.1 Chromatograms of 8 Water-Soluble Vitamins

Here we present an example of analysis of 8 water-soluble vitamins using a cation exchange-anion exchange multi-mode ODS column, (Scherzo SM-C18, Imtakt Corporation) in conjunction with the LCMS2020 mass spectrometric detector. The gradient consisted of formic acid / ammonium formate buffer and acetonitrile mobile phases, components typical for high sensitivity reverse-phase LC/MS analysis. Fig. 1.13.1 shows the chromatograms of the 8 water-soluble vitamins, Fig. 1.13.2 shows the mass spectra, and Fig. 1.13.3 shows the respective calibration curves. The linearity in all cases was excellent, with coefficient of repeatability values all greater than $R^2 = 0.99$.

■Analytical Conditions

Column	: Imtakt Scherzo SM-C18 (150 mm L. × 2.0 mm I.D., 3 µm)
Mobile Phase	: A: 5 mmol/L Ammonium Formate + 0.1 % Formic Acid-Water B: Acetonitrile Gradient Elution Method
Time Program	: B 0 % (0 min) → 55 % (10 min) → 0 % (10.01 - 20 min)
Flowrate	: 0.2 mL/min
Column Temp.	: 40 °C
Injection Volume	: 2 µL
Probe Voltage	: +4.5 kV (ESI-Positive Mode), -3.5 kV (ESI-Negative Mode)
Nebulizing Gas Flow	: 1.5 L/min
Drying Gas Flow	: 10 L/min
DL Temp.	: 250 °C
Block Heater Temp.	: 450 °C
DL/Q-array Voltage	: Using default values

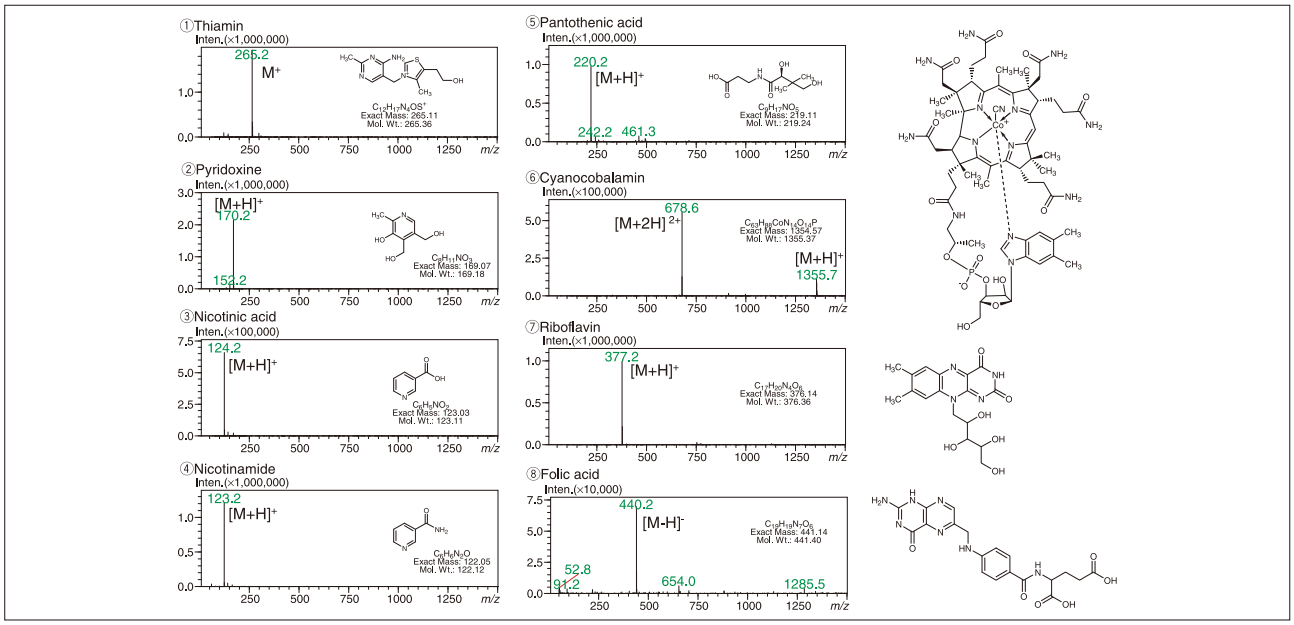


Fig. 1.13.2 Mass Spectra of Water-Soluble Vitamins

1.13 Analysis of Water-Soluble Vitamins with Multi-Mode ODS Column (2) - LC/MS

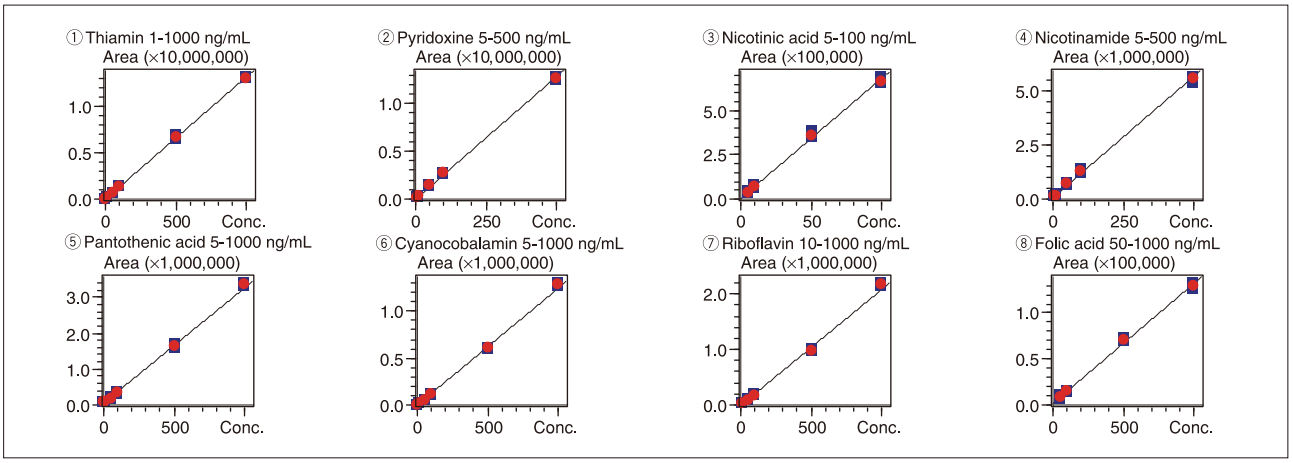


Fig. 1.13.3 Calibration Curves of Water-Soluble Vitamins

■Quantitative Analysis of Water-Soluble Vitamins from Cereal

One gram of a commercial functional food product was prepared according the procedure shown in Fig. 1.13.4, and quantitation of the water-soluble vitamins was conducted. Fig. 1.13.5 shows the SIM chromatograms obtained from analysis of the cereal extract solutions. The content of ⑥ cyanocobalamin (vitamin B_{12}) in the sample was below the method detection limit. Actual sample analysis requires confirmation of extraction efficiency, daily quality control etc., but this analysis confirmed that quantitation was possible without almost any interference from impurities and demonstrated the LCMS-2020 to be a suitably selective and sensitive detector.

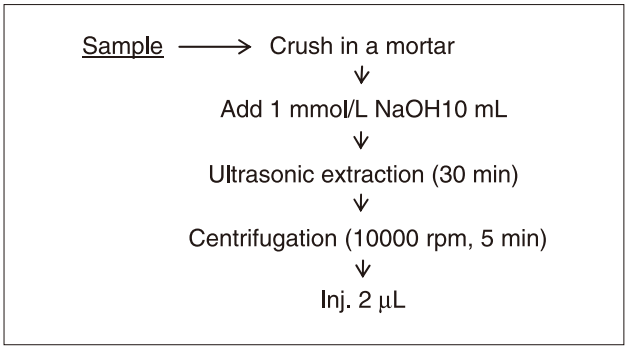


Fig. 1.13.4 Sample Preparation

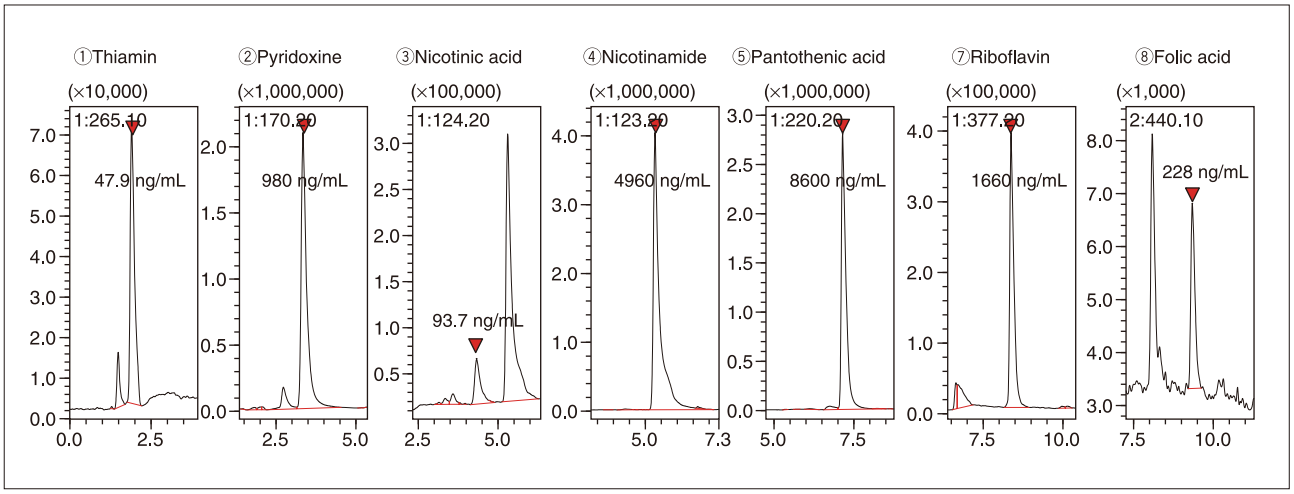


Fig. 1.13.5 SIM Chromatograms of Extract from Cereal

1.14 High Speed Analysis of Nucleobases, Nucleosides, and Nucleotides (1) - LC

■Explanation

Nucleic acids are biological macromolecules consisting of linear chains of nucleotides, each of which is made up of a base, a sugar, and a phosphate group, and are important components that bear an organism's genetic code. In addition, nucleic acid-related compounds, including nucleobases, nucleosides, and nucleotides have a variety of functions. Here, using the Nexera UHPLC (Ultra High Performance Liquid Chromatography) System, and the Shim-pack XR-ODS III and Phenomenex Kinetex C18 high-speed, high-resolution columns, we introduce examples of ultra-high-speed analysis and ultra-high-resolution analysis of nucleic acid-related compounds.

■Analysis of Nucleobases and Nucleosides

We prepared a sample solution consisting of a standard mixture of 10 nucleic acid-related substances, including 5 nucleobases (adenine, guanine, uracil, thymine, cytosine) and 5 nucleosides (adenosine, guanosine, uridine, thymidine, cytidine), each at a concentration of 10 mg/L, and conducted analysis using the Phenomenex Kinetex C18 column (particle size 1.7 µm, 100 mm L. × 2.1 mm I.D.). The Phenomenex Kinetex C18 is a Core-shell column consisting of a 1.25-µm solid core coated with a bonded 0.23 µm multilayer of porous film. Fig. 1.14.1 shows the chromatogram obtained using a 1 µL injection of the prepared standard mixture. This analysis, which took 30 minutes to complete using conventional conditions, took about 1/10 as long (3 minutes) using these analytical conditions. The system back pressure during this analysis was about 75 MPa.

■Analytical Conditions

Column	: Kinetex 1.7 µm C18 100 Å (100 mm L. × 2.1 mm I.D., 1.7 µm)
Mobile Phase	: 200 mmol/L Sodium Perchlorate, 100 mmol/L (Sodium) Phosphate Buffer (pH=2.1) aq.
Flowrate	: 0.7 mL/min
Column Temp.	: 40 °C
Injection Volume	: 1 µL
Detection	: SPD-20AV at 260 nm
Flow Cell	: Semi-micro cell

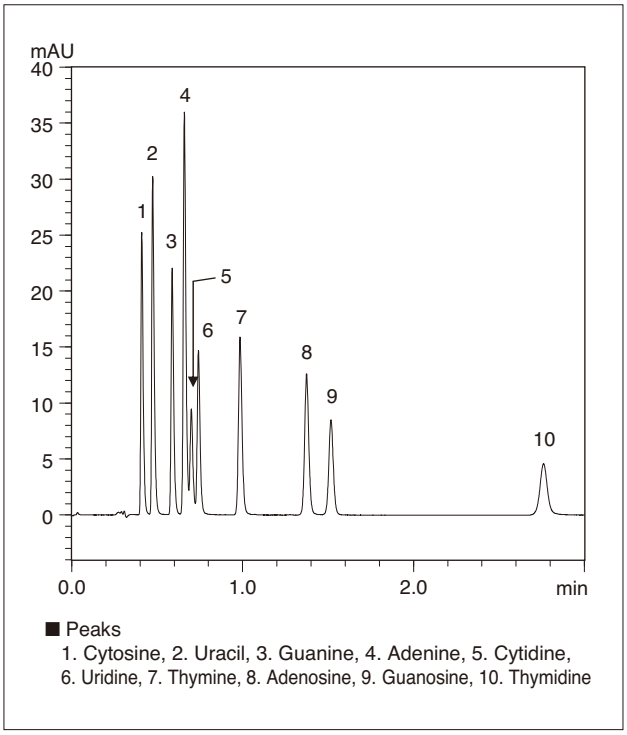


Fig. 1.14.1 Chromatogram of a Standard Mixture of Nucleobases and Nucleosides (10 mg/L each)

Note:
When using a 100 % aqueous mobile phase or a composition close to that, as indicated in the analytical conditions in this document the retention times may become smaller by temporarily stopping solvent delivery, and then restarting. To prevent the occurrence of this phenomenon, after completion of the analysis, it is recommended to replace the mobile phase with one containing an organic solvent (example: water/acetonitrile = 1/1) before stopping solvent delivery. In addition, if the retention times gradually become faster, perform a rinse using the same mobile phase.

1.14 High Speed Analysis of Nucleobases, Nucleosides, and Nucleotides (2) - LC

■Analysis of ATP-related Compounds

We prepared a sample solution consisting of a standard mixture of 6 ATP-related substances (hypoxanthine, inosine, IMP, AMP, ADP, ATP)*, each at a concentration of about 10 mg/L, and conducted analysis using the Shim-pack XR-ODS Ⅲ column (1.6 µm particle size, 50 mm L. × 2.0 mm I.D.). Fig. 1.14.2 shows the chromatogram obtained using a 1 µL injection of the prepared standard mixture.

This analysis, which took 25 minutes to complete using conventional conditions, took about 1/10 as long (2.5 minutes) using these analytical conditions. The system back pressure during this analysis was about 83 MPa.

■Analytical Conditions

Column : Shim-pack XR-ODSⅢ (50 mm L. × 2.0 mm I.D., 1.6 µm)
Mobile Phase : 100 mmol/L Phosphoric Acid, 150 mmol/L Triethylamine aq. / Acetonitrile = 100/1 (v/v)
Flowrate : 0.9 mL/min
Column Temp. : 40 °C
Injection Volume : 1 µL
Detection : SPD-20AV at 260 nm
Flow Cell : Semi-micro cell

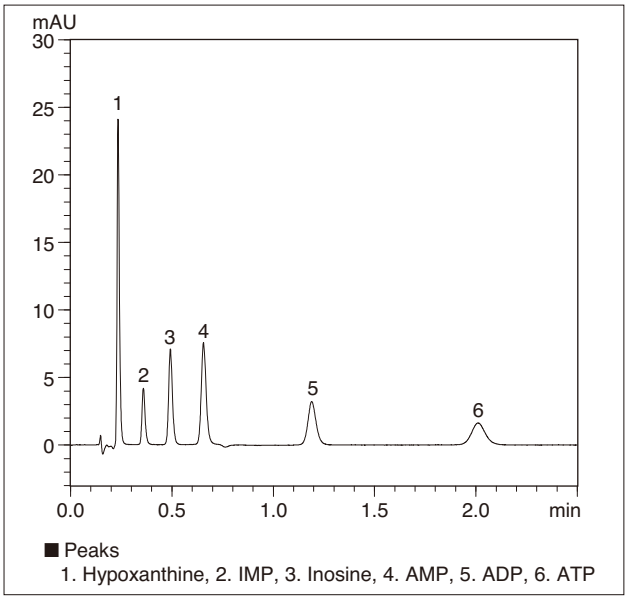


Fig. 1.14.2 Chromatogram of a Standard Mixture of ATP-Related Compounds

■Analysis of Nucleotides

We prepared a sample solution consisting of a standard mixture of 18 nucleotides (AMP, ADP, ATP, GMP, GDP, GTP, UMP, UDP, UTP, TMP, TDP, TTP, CMP, CDP, CTP, IMP, IDP, ITP)*, each at a concentration of about 50 mg/L, and conducted analysis using the high-resolution Shim-pack XR-ODSⅢcolumn (2.2 µm particle size, 200 mm L. × 2.0 mm I.D.). Fig. 1.14.3 shows the chromatogram obtained using a 1 µL injection of the prepared standard mixture. Analysis of these 18 substances was achieved at high speed and with high resolution using these conditions, and the system back pressure during the analysis was about 78 MPa.

■Analytical Conditions

Column : Shim-pack XR-ODS Ⅲ (200 mm L. × 2.0 mm I.D., 2.2 µm)
Mobile Phase : A: 100 mmol/L Phosphoric Acid, 150 mmol/L Triethylamine aq.
B: Mobile Phase A / Acetonitrile = 90/10 (v/v)
A/B = 98/2 (v/v)
Flowrate : 0.6 mL/min
Column Temp. : 50 °C
Injection Volume : 1 µL
Detection : SPD-20AV at 260 nm
Flow Cell : Semi-micro cell

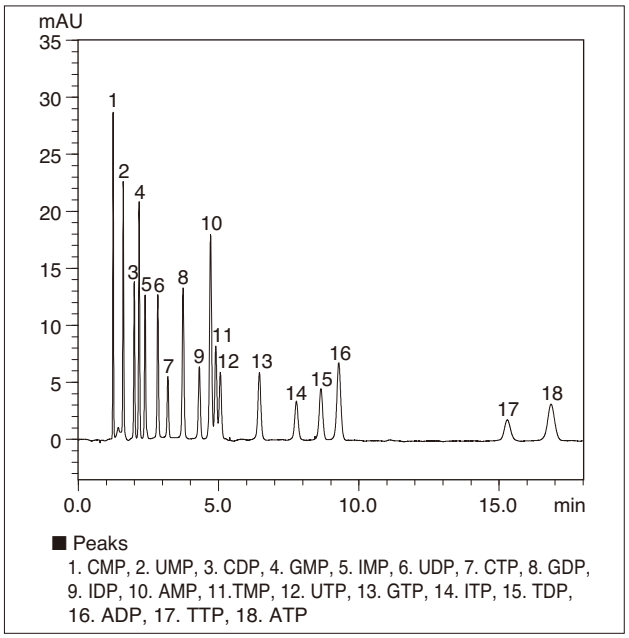


Fig. 1.14.3 Chromatogram of a Standard Mixture of Nucleotides

* AMP: Adenosine 5'-monophosphate, ADP: Adenosine 5'-diphosphate, ATP: Adenosine 5'-triphosphate, GMP: Guanosine 5'-monophosphate, GDP: Guanosine 5'-diphosphate, GTP: Guanosine 5'-triphosphate, UMP: Uridine 5'-monophosphate, UDP: Uridine 5'-diphosphate, UTP: Uridine 5'-triphosphate, TMP: Thymidine 5'-monophosphate, TDP: Thymidine 5'-diphosphate, TTP: Thymidine 5'-triphosphate, CMP: Cytidine 5'-monophosphate, CDP: Cytidine 5'-diphosphate, CTP: Cytidine 5'-triphosphate, IMP: Inosine 5'-monophosphate, IDP: Inosine 5'-diphosphate, ITP: Inosine 5'-triphosphate

1.15 Analysis of Oligosaccharides in Beer - LC

■Explanation

Combining the gradient elution method with the ELSD-LT enables efficient separation when analyzing oligosaccharides. Fig. 1.15.1 shows the result of analyzing oligosaccharides in beer using the isocratic elution method and the gradient elution method. 10 µL of beer was injected after filtering with a membrane filter. There are branched oligosaccharides (1→6 glycosidic linkage), linear oligosaccharides (1→4 glycosidic linkage), and other types oligosaccharides. In general, the different types of oligosaccharide are mixed together when eluted. The elution times for monosaccharides as well as linear disaccharides, trisaccharides, and heptasaccharides are indicated in the chromatogram. It can be seen that the gradient elution method enables the efficient separation and detection of oligosaccharides up to 20-mer. The results of analyzing commercial beers under the same gradient elution conditions are shown in Fig. 1.15.2 and Fig. 1.15.3.

■Analytical Conditions

Column : Asahipak NH2P - 50 4E
(250 mm L. × 4.6 mm I.D.)
Mobile Phase : (1) Acetonitrile / Water = 6/4 (v/v %)
(Fig. 1.15.1)
(2) A: Acetonitrile
B: Water
Linear Gradient B 30 % → 60 %
(Fig. 1.15.2 and Fig. 1.15.3)
Flowrate : 1.0 mL/min
Column Temp. : 40 °C
Detection : ELSD-LT
Temperature : 35 °C
GAIN : 7
Nebulizer Gas : N_2
Gas Pressure : 350 kPa

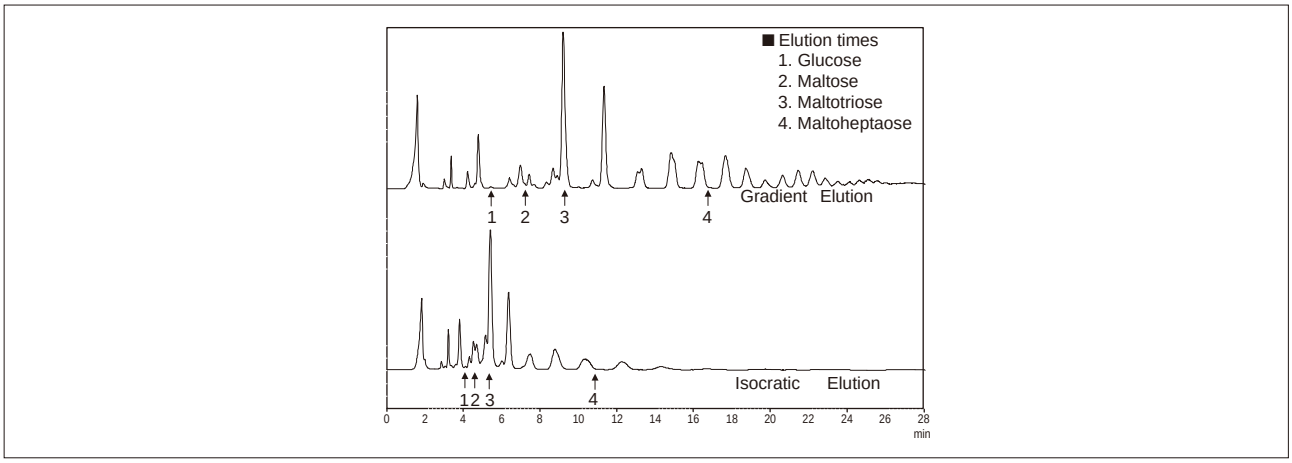


Fig. 1.15.1 Analysis of Oligosaccharides in Beer

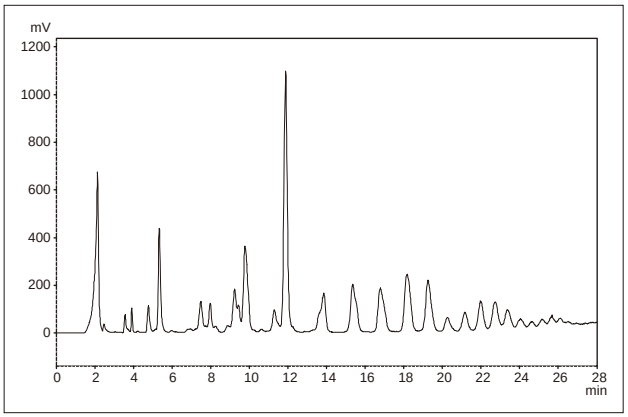


Fig. 1.15.2 Chromatogram of beer A

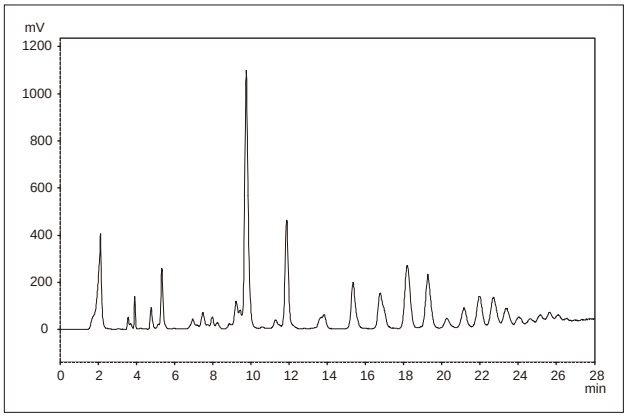


Fig. 1.15.3 Chromatogram of beer B

1.16 Determination of Fructo-oligosaccharides in Food - LC

■Explanation

Fructo-oligosaccharides are types of oligosaccharides consisting of fructose and glucose. They have lower calories than sucrose, and recently, have received attention related to their contribution to the proliferation of bifidobacteria, possibly because they reach the large intestine without decomposition by enzymes. Recently, many food products with fructo-oligosaccharides have reached the market. Here we introduce the simultaneous analysis of sugars and fructo-oligosaccharides in food using HPLC.

■Analytical Conditions

Column	: Asahipak NH2P-50 4D (150 mm L. × 4.6 mm I.D.)
Guard Column	: Asahipak NH2P-50G (10 mm L. × 4.6 mm I.D.)
Mobile Phase	: Water / Acetonitrile = 3/7 (v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 30 °C
Detection	: Refractive Index Detector Polarity +, Cell temp. 40 ˚C

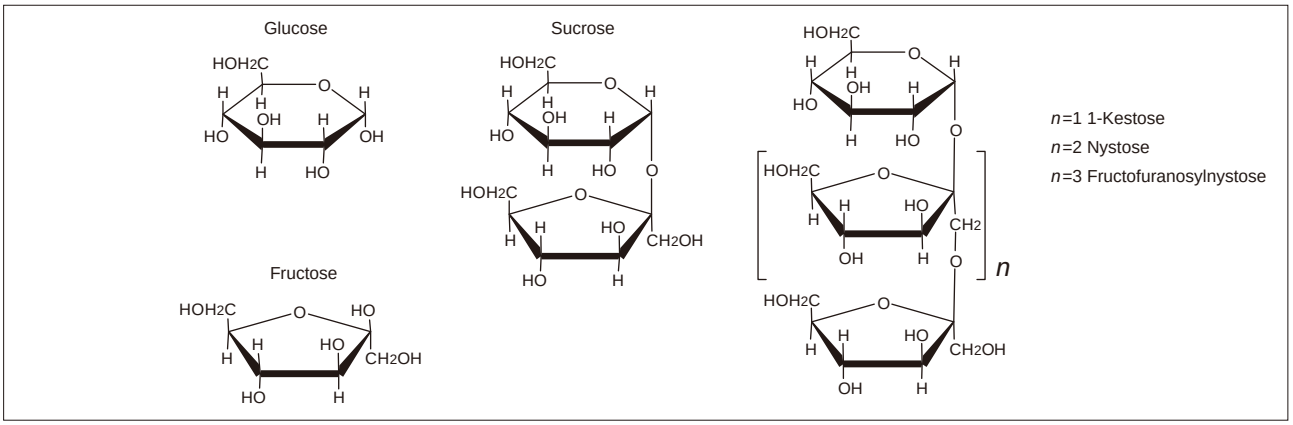


Fig. 1.16.1 Structural Formulas

■Analysis of Standard Solution

Fig. 1.16.1 shows the structural formulas of sucrose, fructo-oligosaccharides kestose, nystose and fructofuranosylnystose, as well as glucose and fructose, which are the components of these sugars. The chromatogram obtained from analysis of a standard mixture of these six compounds (each 5.0 g/L) is shown in Fig. 1.16.2.

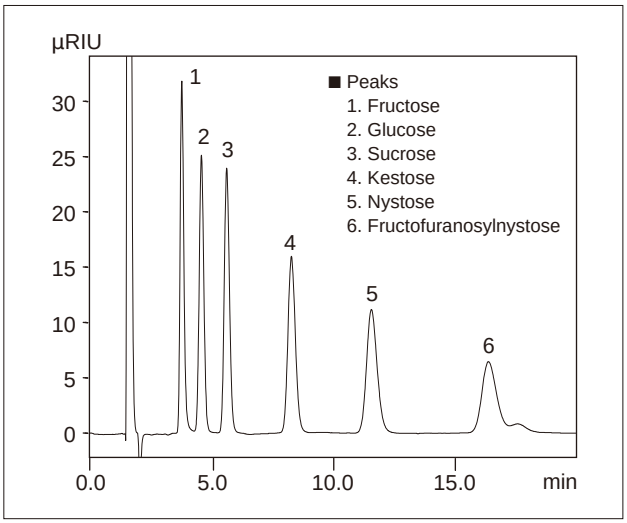


Fig. 1.16.2 Chromatogram of a Standard Mixture of Fructo-oligosaccharides (5.0 g/L each, 10 μL injected)

■Analysis of Syrup

Fig. 1.16.3 shows the chromatogram of a commercially available syrup containing fructo-oligosaccharides. 1.0 g of the syrup was diluted and made 200 mL with distilled water. This solution was then filtered using a 0.45 μm pore membrane filter.

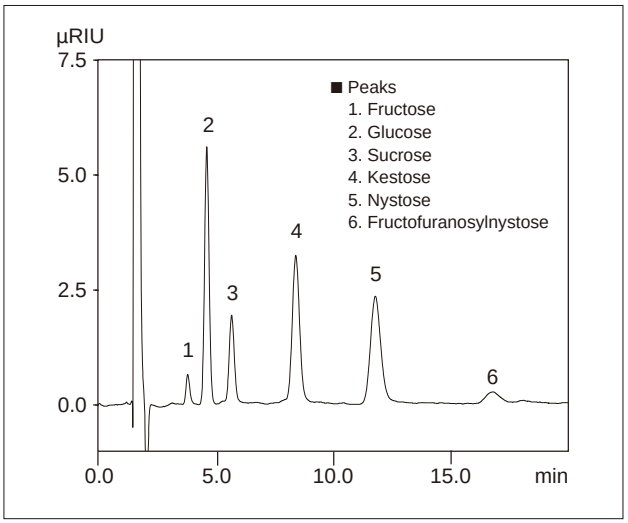


Fig. 1.16.3 Chromatogram of Oligosaccharide Syrup (10 μL injected)

1.17 Analysis of Saccharides Using Post-Column Derivatization System (1) - LC

■Explanation

The Shimadzu Prominence reducing sugar analysis system is a post-column derivatization system; after separation of saccharides by the column, an arginine/boric acid reagent solution is continuously added to the column eluent to convert the saccharides to fluorescent derivatives for detection. This system allows detection of saccharides at high sensitivity and with excellent selectivity, furthermore with the new Prominence RF-20Axs , even higher sensitivity is achieved. Additionally, the temperature controlled flowcell in the RF-20Axs allows highly reliable analysis that is unaffected by ambient temperature fluctuations. Here we present some examples of analyzing saccharides using the reducing sugar analysis system (anion exchange mode) with the Prominence RF-20Axs .

■Analysis of Standard Solution

Fig. 1.17.1 shows a flow diagram of the Prominence reducing sugar analysis system, and Fig. 1.17.2 shows the chromatogram of a standard solution of 11 saccharides.

■Analytical Conditions

<Separation>

Column : Shim-pack ISA-07/S2504 (250 mm L. × 4.0 mm I.D.)
Guard Column : Shim-pack Guard Column ISA (50 mm L. × 4.0 mm I.D.)
Mobile Phase : A: 0.1 mol/L Potassium Borate Buffer (pH 8)
B: 0.4 mol/L Potassium Borate Buffer (pH 9)
A → B Linear Gradient Elution
Flowrate : 0.6 mL/min
Column Temp. : 65 °C
Injection Volume : 10 µL

<Detection>

Reaction Reagent : 10 g/L Arginin, 30 g/L Boric Acid
Flowrate : 0.5 mL/min
Reaction Coil : SUS, 10 m L. × 0.8 mm I.D.
Reaction Temp. : 150 ˚C
Cooling Coil : SUS, 6 m L. × 0.3 mm I.D.
Detection : RF-20Axs Ex. at 320 nm, Em. at 430 nm
Cell Temp. : 25 ˚C

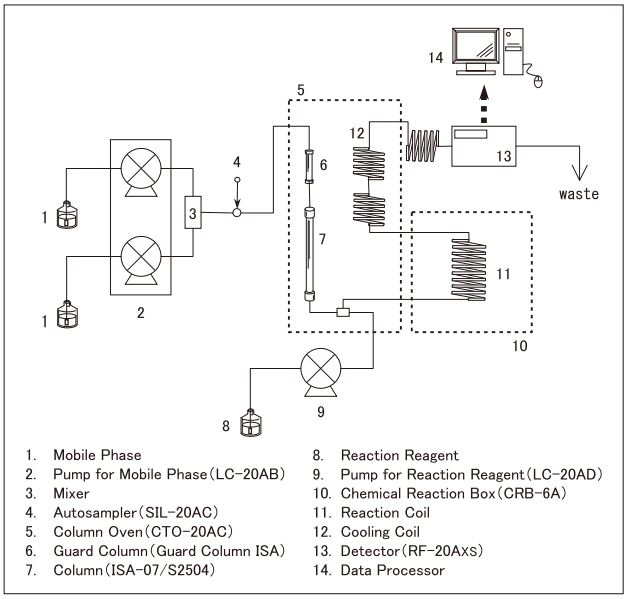


Fig. 1.17.1 Flow Diagram of the System

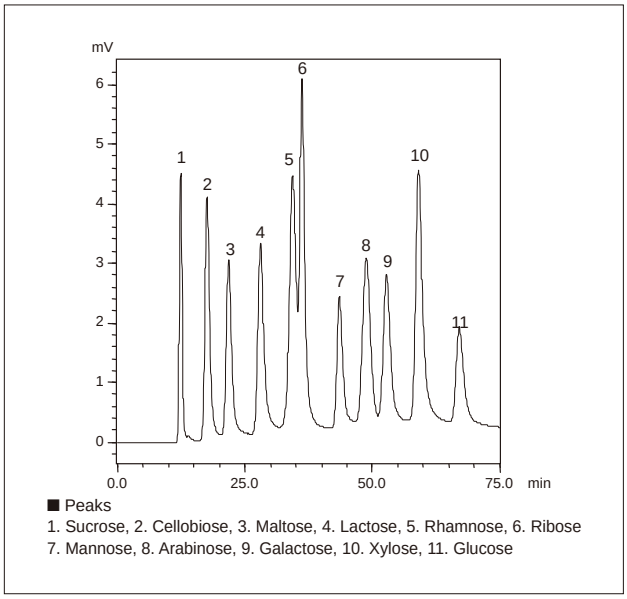


Fig. 1.17.2 Chromatogram of a Standard Mixture of 11 Saccharides (200 µmol/L each, except sucrose at 2 mmol/L, 10 µL injected)

1.17 Analysis of Saccharides Using Post-Column Derivatization System (2) - LC

■High-Sensitivity Analysis

The RF-20Axs offers unprecedented levels of sensitivity; a water Raman S/N ratio is at least 2000. This allows the reducing sugar analysis system to detect saccharides with much higher sensitivity. Fig. 1.17.3 shows a chromatogram obtained from analysis of a standard solution of saccharides (2 µmol/L each, except sucrose at 20 µmol/L, 10 µL injected). In this case, glucose is clearly detected even when injected at the amount of 20 pmol (3.6 ng).

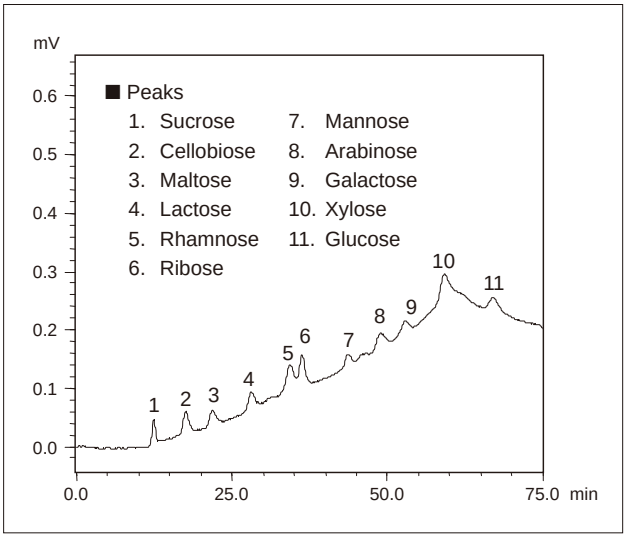
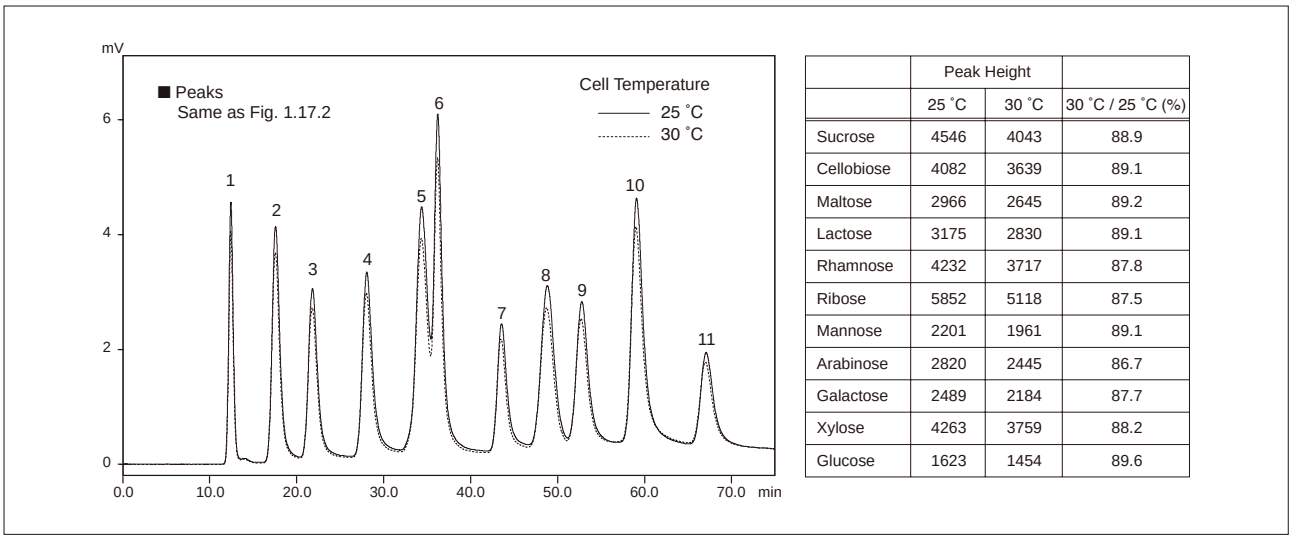


Fig. 1.17.3 Chromatogram of a Standard Mixture of 11 Saccharides (2 µmol/L each, except sucrose at 20 µmol/L, 10 µL injected)

■Effect of Cell Temperature Control

It is generally known that the fluorescence intensity drops as the temperature rises; because the molecular collisions increase in frequency with increase in the temperature, therefore molecules lose their potential energy. In other words, a fluctuation of the ambient (detection) temperature changes the fluorescence intensity of some compounds, and this negatively influences the accuracy of analysis. The RF-20Axs has a temperature-controlled cell as a standard feature, ensuring the high reliability of analysis that is not affected by a temperature fluctuation. Fig. 1.17.4 shows a comparison of the peak intensities at cell temperatures of 25 °C and 30 °C (using the same saccharide solution and analytical conditions as in Fig. 1.17.2). The comparison of two chromatogram at cell temperatures of 25 °C and 30 °C reveals a decrease in peak intensity over 10 % for every compound at the higher cell temperature. By maintaining a constant cell temperature, peak intensity and detection sensitivity will not be compromised if the room temperature changes during the sequence run.



	Peak Height		
	25 ˚C	30 ˚C	30 ˚C / 25 ˚C (%)
Sucrose	4546	4043	88.9
Cellobiose	4082	3639	89.1
Maltose	2966	2645	89.2
Lactose	3175	2830	89.1
Rhamnose	4232	3717	87.8
Ribose	5852	5118	87.5
Mannose	2201	1961	89.1
Arabinose	2820	2445	86.7
Galactose	2489	2184	87.7
Xylose	4263	3759	88.2
Glucose	1623	1454	89.6

Fig. 1.17.4 Effect of Cell Temperature on Peak Intensity

1.18 Analysis of Ethyl α-D-Glucoside in Japanese Sake - LC

■Explanation

Japanese sake typically contains ethyl α-D-glucoside (α-EG), a substance believed to promote beautiful skin, and it is now receiving attention for its effectiveness in improving dry skin conditions even when taken in a drink. Here we present an example of analysis of ethyl α-D-glucoside and other saccharides in sake using hydrophilic interaction liquid chromatography (HILIC).

■Analysis of Standard Solution

Fig. 1.18.1 shows the structural formula of ethyl α-D-glucoside (α-EG). α-EG is a highly polar compound, so by using hydrophilic interaction liquid chromatography (HILIC) α-EG and other saccharides can be analyzed at the same time. Since α-EG shows almost no UV absorption, common with other saccharides, in addition to the refractive index detector used for this analysis, an evaporative light-scattering detector can also be used. Fig. 1.18.2 shows the chromatogram obtained from analysis of a standard solution of ethyl α-D-glucoside and other saccharides (fructose, glucose, sucrose, and maltose, each at 20 g/L).

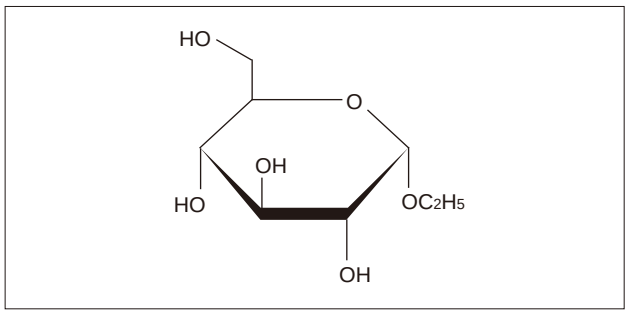


Fig. 1.18.1 Structure of Ethyl α-D-Glucoside

■Analytical Conditions

Column	: Asahipak NH2P - 50 4E (250 mm L. × 4.6 mm I.D.)
Mobile Phase	: Water / Acetonitrile = 30/70
Flowrate	: 0.8 mL/min
Column Temp.	: 30 °C
Injection Volume	: 10 µL
Detection	: Refractive Index Detector

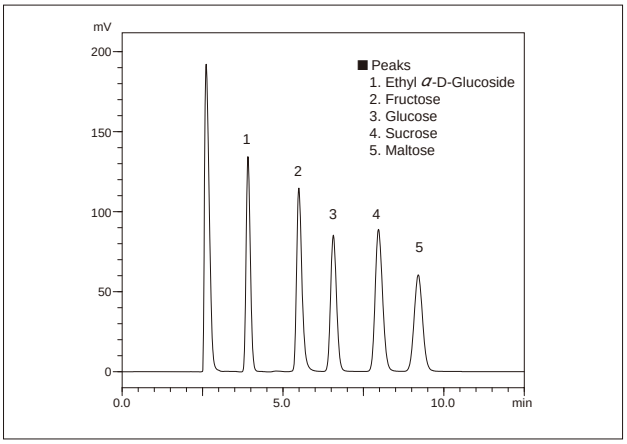


Fig. 1.18.2 Chromatogram of a Standard Mixture of Ethyl α-D-Glucoside and 4 Saccharides (20 g/L each, 10 µL injected)

■Analysis of Sake (Alcoholic Beverage from Rice)

Fig. 1.18.3 and Fig. 1.18.4 show chromatograms of commercial sake. In both cases, simultaneous analysis was conducted for α-EG and glucose, the primary saccharide ingredient of sake. After filtering the sake through a 0.45 µm membrane filter, 10 µL was injected for each analysis.

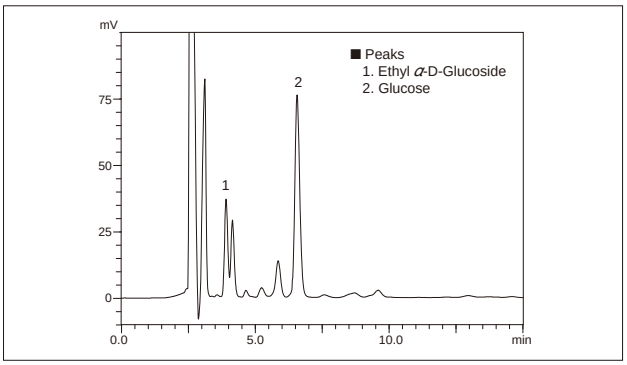


Fig. 1.18.3 Chromatogram of Sake A (10 µL injected)

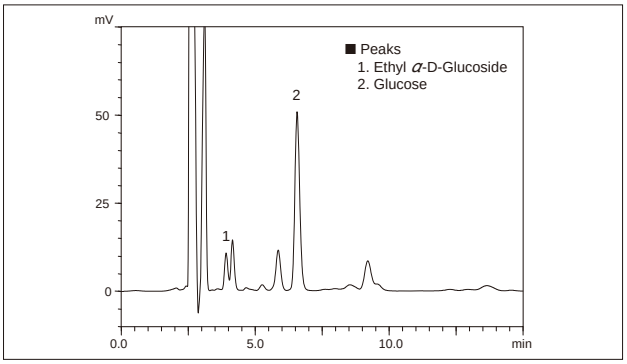


Fig. 1.18.4 Chromatogram of Sake B (10 µL injected)

1.19 Analysis of Alliin in Garlic - LC

■Explanation

The active ingredient alliin in garlic quickly changes to allicin by the action of the enzyme alliinase. As the efficacy of these substances has been elucidated, they are often marketed as health food products. Since alliin is a type of amino acid, it can be analyzed by the post-column derivatization method with fluorescence detection using OPA (*o*-phthalaldehyde) as the reaction reagent. Here we introduce the analysis of alliine in garlic using the Shimadzu amino acid analysis system.

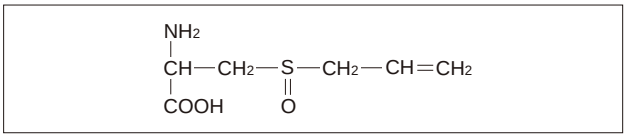


Fig. 1.19.1 Structure of Alliin

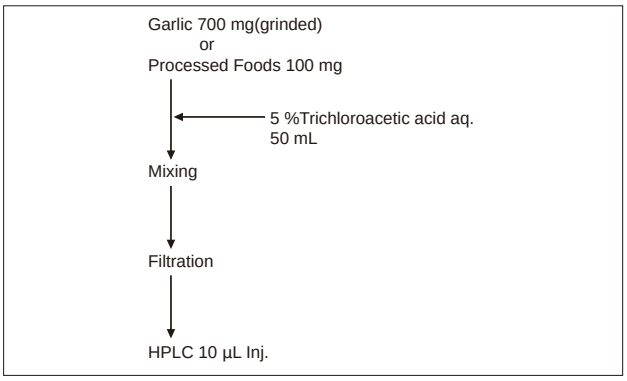


Fig. 1.19.2 Sample Preparation

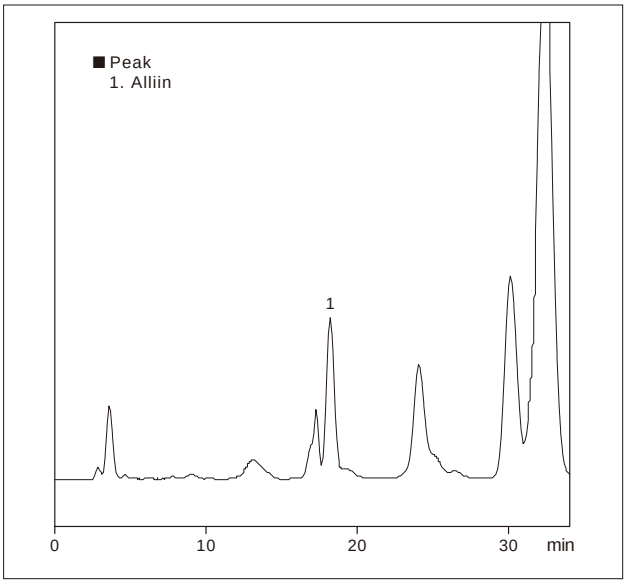


Fig. 1.19.3 Analysis of Garlic

■Analytical Conditions

Column	: Shim-pack Amino-Na (100 mm L. × 6.0 mm I.D.)
Mobile Phase	: A: 0.1 mol/L Sodium Citrate Buffer Solution (pH 3.2) B: 0.2 mol/L Sodium Hydroxide Aqueous Solution Step Gradient Elution Method 0 – 23 (min) A 100 % 23 – 33 (min) B 100 % 33 – 50 (min) A 100 %
Mobile Phase Flowrate	: 0.4 mL/min
Column Temp.	: 60 °C
Reaction Reagent	: Amino Acid Reagent Kit (Solution B)
Reaction Reagent Flowrate	: 0.4 mL/min
Reaction Temp.	: 60 °C
Detection	: Fluorescence Detector Ex: 350 nm Em: 450 nm
Injection Volume	: 10 µL

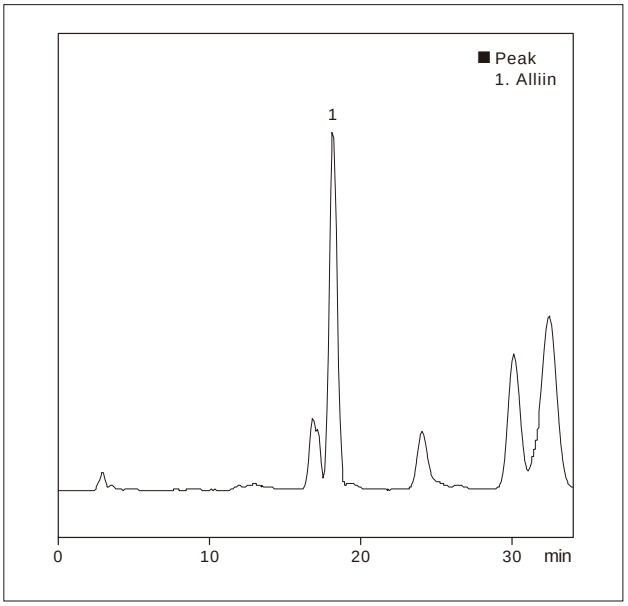


Fig. 1.19.4 Analysis of Processed Garlic (tablet)

1.20 High Speed Analysis of Catechins in Green Tea (1) - LC

■Explanation

Along with the health consciousness boom that has been ongoing in recent years, green tea and green tea drinks have been receiving heightened attention.

Here we introduce an example of high-speed analysis of 14 catechins in green tea, including methyl catechins, which have been reported to display anti-allergic action. The analytical system consisted of the Prominence UFLCXR ultra-fast, high-resolution LC system with a high-speed, high-resolution column. In addition, detection was also conducted using the LCMS-2020, and these results were compared to those obtained using UV detection.

■Analysis of Standard Solution

Fig. 1.20.1 shows the structures of the catechins analyzed here, including methyl catechins, gallic acid and caffeine. Fig. 1.20.2 shows a chromatogram of a standard mixture of these14 substances (100 mg/L each), which were analyzed all together with a high-speed and high-resolution HALO® C18 column (particle size 2.7 µm) from AMT Ltd.

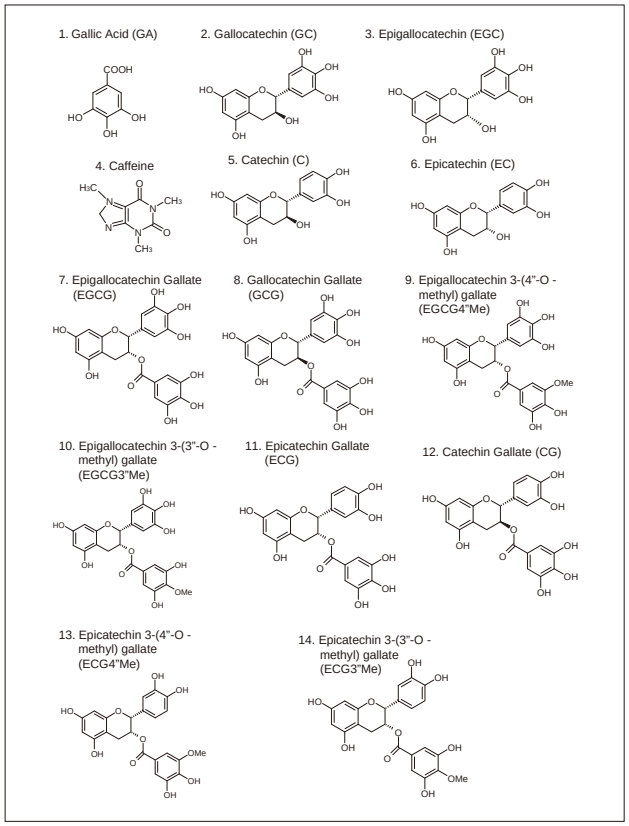


Fig. 1.20.1 Structures of Catechins, Gallic Acid and Caffeine

■Analytical Conditions

Column	: HALO® C18 (75 mm L. × 3.0 mm I.D., 2.7 µm)
Mobile Phase	: A: 0.2 % Phosphoric Acid (100 %) in Water / Tetrahydrofuran = 995/5 (v/v)
	B: Acetonitrile / Tetrahydrofuran = 990 / 10 (v/v)
	Gradient Elution Method
Time Program	: B 10 % (0 min) → 15 % (2.5 min) → 50 % (2.51-3.0 min)
Flowrate	: 2.0 mL/min
Column Temp.	: 40 °C
Injection Volume	: 2 µL
Detection	: SPD-M20A at 230 nm
UV Cell	: Semi-micro cell

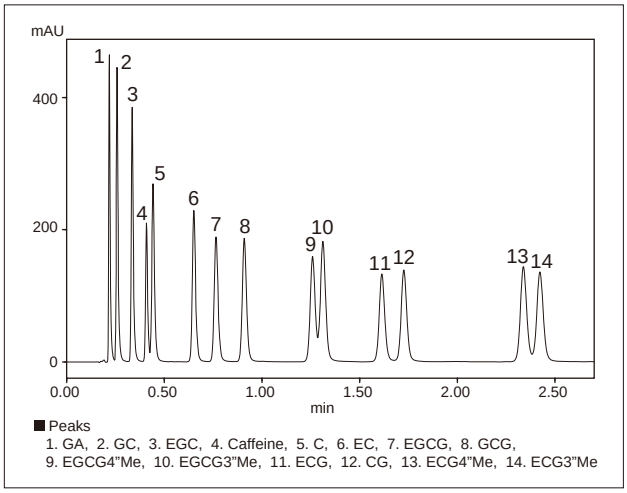


Fig. 1.20.2 Chromatogram of a Standard Mixture of Catechins, Gallic Acid and Caffeine (100 mg/L, 2 µL injected)

1.20 High Speed Analysis of Catechins in Green Tea (2) - LC

■Analysis of Green Tea

Fig. 1.20.3 and Fig. 1.20.4 show the chromatograms of commercial green teas A and B, respectively. The green tea leaves (3 g per sample) were infused in 100 mL of hot water, diluted by a factor of 10 with purified water and the diluted infusion was passed through a membrane filter (pore size 0.22 µm). The methyl catechins EGCG3" Me and ECG3" Me were confirmed to be present at different concentrations, respectively, in green teas A and B.

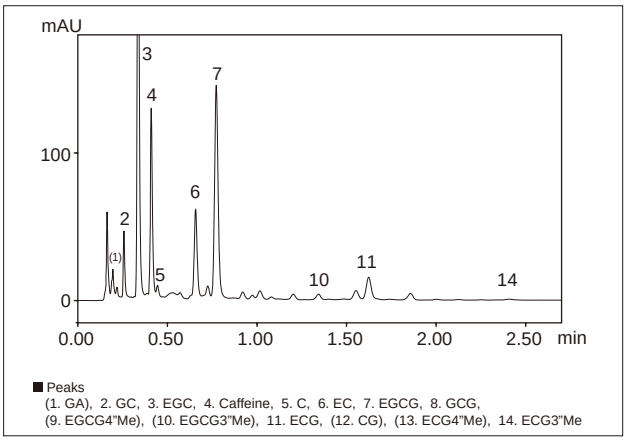


Fig. 1.20.3 Chromatogram of Green Tea A

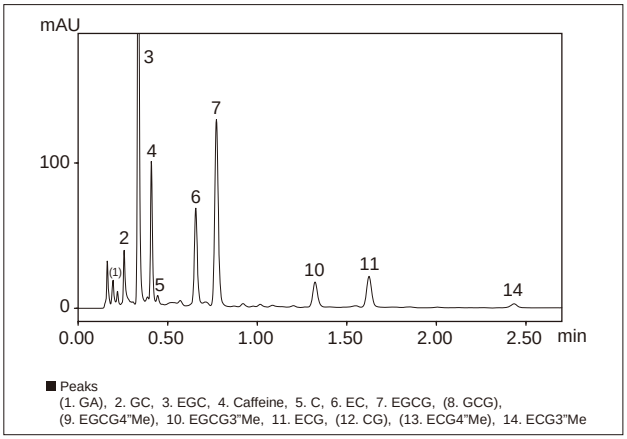


Fig. 1.20.4 Chromatogram of Green Tea B

■Analysis of Green Tea by LC-MS

Fig. 1.20.5 shows the MS chromatogram of green tea B analyzed using the LCMS-2020. The acid added to the mobile phase for the LC-MS measurement was changed to the volatile formic acid, and it was also added to the tetrahydrofuran in Solvent B. (Since a different mobile phase than that used with UV detection was used in the LC-MS method, the order of elution of peak 4 (C) and peak 5 (caffeine) was observed to be reversed. In addition, GA, which was observed in Fig. 1.20.4, was not confirmed in the LC-MS analysis results of Fig. 1.20.5, suggesting that is may be a compound other than GA.

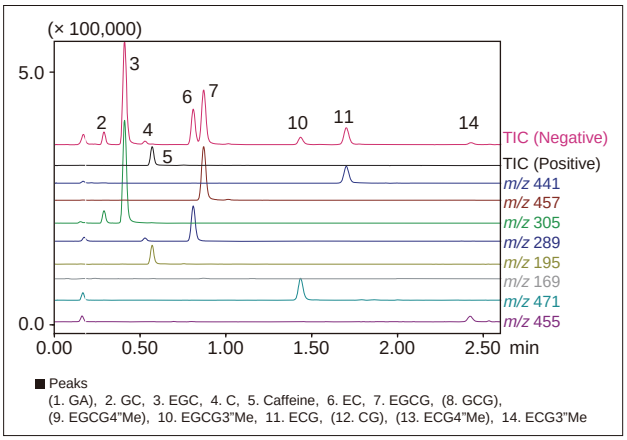


Fig. 1.20.5 MS Chromatograms of Green Tea B

■Analytical Conditions

■LC Conditions

Column	: HALO® C18 (75 mm L. × 3.0 mm I.D., 2.7 µm)
Mobile Phase	: A: 0.4 % Formic Acid in Water / Tetrahydrofuran = 995/5 (v/v) B: 0.4 % Formic Acid in Tetrahydrofuran / Acetonitrile = 10/990 (v/v) Gradient Elution Method
Time Program	: B 8 % (0 min) → 15 % (2.5 min) → 50 % (2.51-3.0 min)
Flowrate	: 2.0 mL/min
Column Temp.	: 40 °C
Injection Volume	: 2 µL

■MS Conditions (LCMS-2020)

Probe Voltage	: +4.5 kV (ESI Positive Mode), -3.5 kV (ESI Negative Mode)
Nebulizing Gas Flow	: 1.5 L/min.
Drying Gas Flow	: 20 L/min.
DL Temp.	: 250 °C
DL/Q-array Voltage	: Using default values
Monitoring Ions	: *m/z* 441 (Negative) for ECG and CG *m/z* 457 (Negative) for EGCG and GCG *m/z* 305 (Negative) for GC and EGC *m/z* 289 (Negative) for C and EC *m/z* 195 (Positive) for Caffeine *m/z* 169 (Negative) for GA *m/z* 471 (Negative) for EGCG4" Me and EGCG3" Me *m/z* 455 (Negative) for ECG4" Me and ECG3" Me

1.21 High Speed Analysis of Resveratrol in Wine - LC

■Explanation

Resveratrol, a type of polyphenol, is a phytoalexin that is synthesized by plants when they are exposed to stress due to disease and pests. In addition to providing antioxidative effects, resveratrol is reported to offer other health benefits including support for increased longevity. For these reasons, this substance is actively being researched, and recently, resveratrol is being added to food products and to cosmetics. Here we introduce an example of ultra fast analysis of resveratrol in red wine using the Nexera ultra high performance liquid chromatograph and the Prominence RF-20Axs high-sensitivity fluorescence detector.

■Analysis of Standard Solution

Fig. 1.21.1 shows the structural formulas of *cis*- and *trans*-resveratrol. Fluorescence detection can be used for both forms. Fig. 1.21.2 shows the results of measurement of a standard mixture of *trans*- and *cis*-resveratrol* (25 mg/L each in 50 % methanol). The system pressure during this analysis was 75 MPa (10,900 psi) at maximum, while the pressure tolerance of the Shim-pack XR-ODS Ⅲ column (2.2 µm particle size) is rated at 100 MPa (14,500 psi).

* The *cis*- form of the resveratrol standard contains a small amount of the *trans*- isomer.

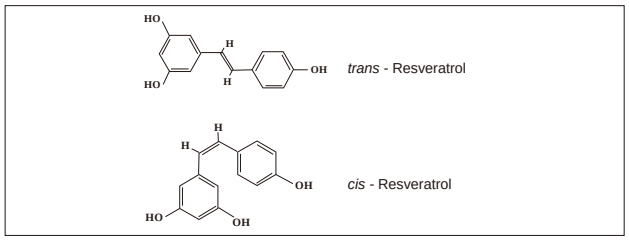


Fig. 1.21.1 Structures of *cis*- and *trans*-Resveratrol

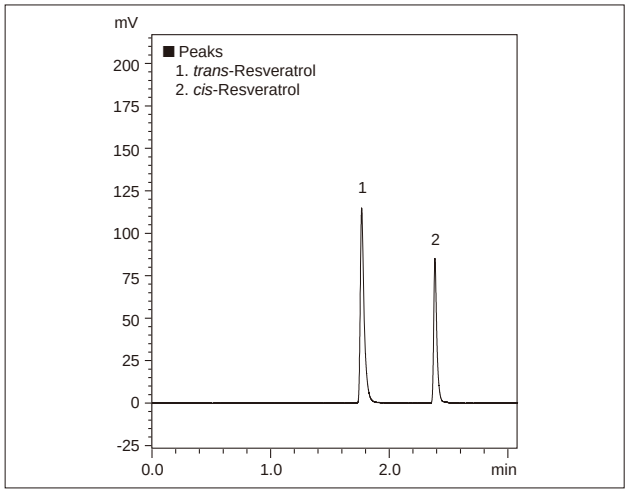


Fig. 1.21.2 Chromatogram of a Standard Mixture of *cis*- and *trans*-Resveratrol (25 mg/L each, 1 µL injected)

■Analytical Conditions

Column	: Shim-pack XR-ODS Ⅲ (150 mm L. × 2.0 mm I.D., 2.2 µm)
Mobile Phase	: A: 0.2 % Formic Acid - Water B: 0.2 % Formic Acid - Acetonitrile Gradient Elution Method
Time Program	: B 23 % (0.00 min) → 26 % (1.00 min) → 40 % (2.50 min) → 100 % (2.51-4.00 min) → 23 % (4.01 min)
Flowrate	: 0.7 mL/min
Column Temp.	: 60 °C
Injection Volume	: 1 µL
Detection	: RF-20Axs Ex. at 300 nm, Em. at 386 nm
Cell Temp.	: 20 °C
Flow Cell	: Semi-micro cell

■Analysis of Red Wine

Fig. 1.21.3 shows the sample preparation procedure, and Fig. 1.21.4 shows the chromatogram obtained from analysis of red wine prepared using this procedure.

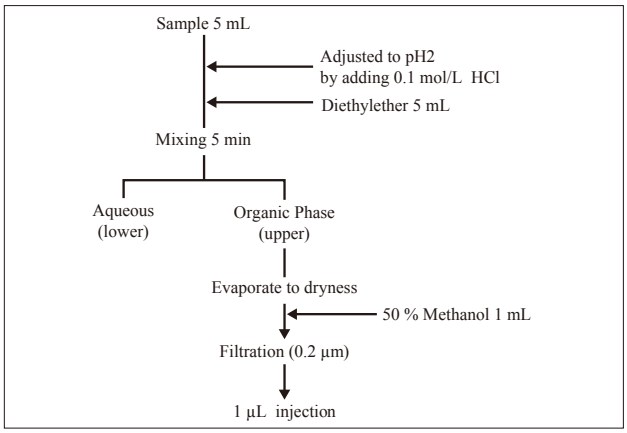


Fig. 1.21.3 Sample Preparation

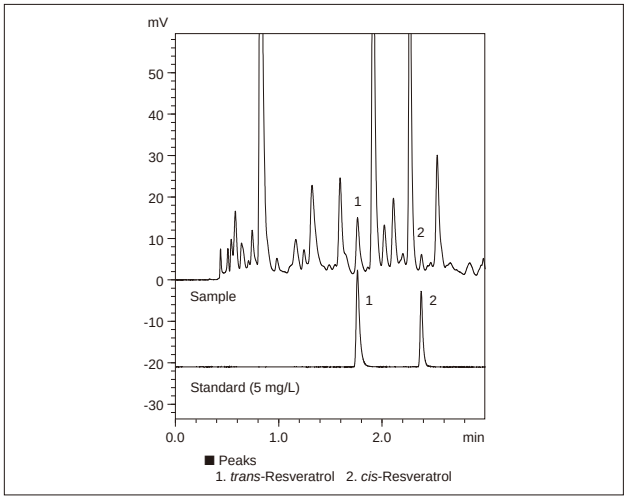


Fig. 1.21.4 Chromatogram of Red Wine

1.22 High Speed Analysis of Phenolic Acids (1) - LC

■Explanation

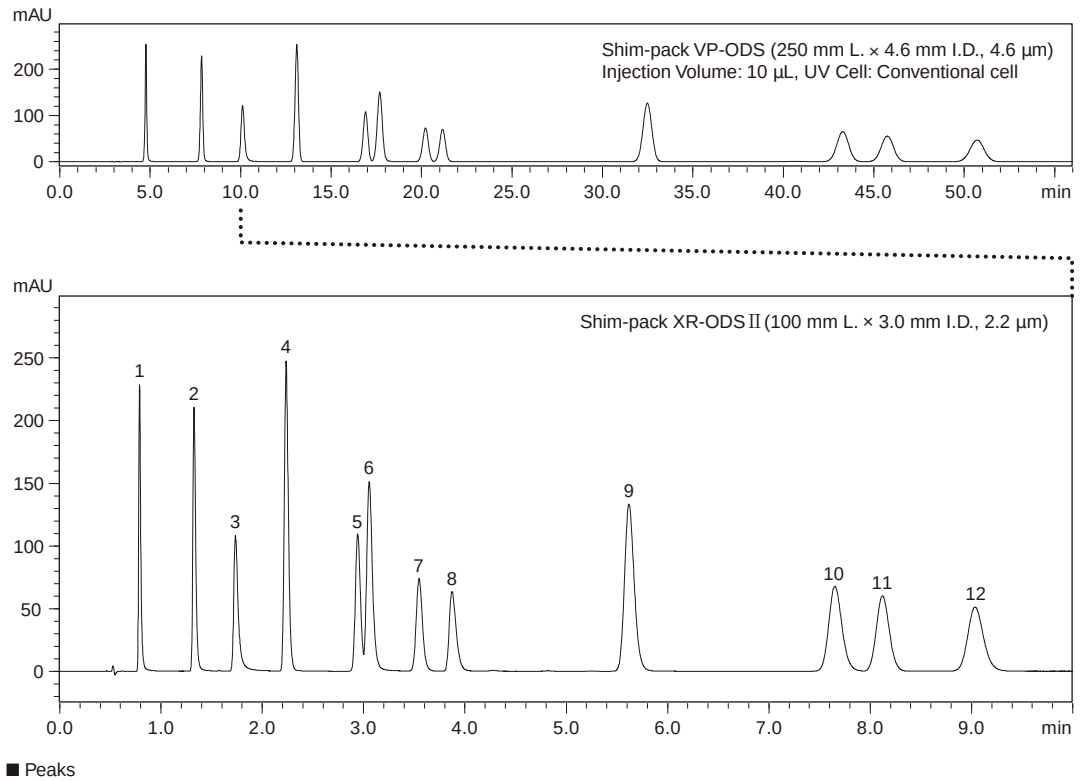
Phenolic acid exists in higher plants as esters, ethers or in its free state, and is a compound that has been receiving increased attention in recent years due to its antioxidant effects. HPLC is often used for quantitative analysis of phenolic acid in processed foods containing fruits and fruit materials, but the analysis is often quite time-consuming because a relatively long column and gradient elution are required to achieve separation of the contaminants in actual samples. Here we present an example of high speed, high resolution analysis of phenolic acids using the Shimadzu Prominence UFLCXR ultra high-speed LC system with the SPD-M20A photodiode array detector.

■Simultaneous Analysis of 11 Phenolic Acids and Benzoic Acid

Fig. 1.22.1 shows a chromatogram of a standard mixture of 11 phenolic acids and benzoic acid (50 mg/L each), analyzed using a Shim-pack XR-ODS II high speed, high resolution column. For comparison, data acquired using a conventional Shim-pack VP-ODS column are also shown.

■Analytical Conditions

Column	: Shim-pack XR-ODS II (100 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile Phase	: A: 50 mmol/L Ammonium Formate Buffer (pH 3.6) B: Methanol Gradient Elution Method
Time Program	B 20 % (0-10 min) → 80 % (10.01-11 min) → 20 % (11.01-15 min) 0.5 mL mixer
Flowrate	: 0.9 mL/min
Column Temp.	: 40 °C
Injection Volume	: 4 µL
Detection	: SPD-M20A (Max plot 230-350 nm)
UV Cell	: Semi-micro cell



■ Peaks
1. Gallic acid, 2. Protocatechuic acid, 3. Chlorogenic acid, 4. *p*-Hydroxybenzoic acid, 5. Vanillic acid, 6. Caffeic acid, 7. Syringic acid
8. Salicylic acid, 9. *p*-Coumaric acid, 10. Ferulic acid, 11. Benzoic acid, 12. Sinapic acid

Fig. 1.22.1 Chromatograms of a Standard Mixture of 11 Phenolic Acids and Benzoic Acid (50 mg/L each)

1.22 High Speed Analysis of Phenolic Acids (2) - LC

■Analysis of Chlorogenic Acid

Here we present a high speed analysis of chlorogenic acid in commercially available fruit juices.

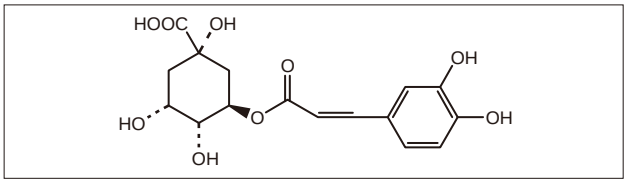


Fig. 1.22.2 Structure of Chlorogenic Acid

■Analytical Conditions

Column	: Shim-pack XR-ODS (50 mm L. × 3.0 mm I.D., 2.2 μm)
Mobile Phase	: A: 50 mmol/L Ammonium Acetate Buffer (pH 4.7) B: Methanol Gradient Elution Method
Time Program	: B 15 % (0-0.8 min) → 80 % (0.81-1.0 min) → 15 % (1.01-1.8 min)
Flowrate	: 0.9 mL/min
Column Temp.	: 40 °C
Injection Volume	: 4 μL
Detection	: SPD-M20A (325 nm)
UV Cell	: Semi-micro cell

■Analysis of Ellagic Acid

Ellagic acid is found in many plants, in which much of it exists in the form of tannin combined with gallic acid. Here we analyzed free ellagic acid in processed food.

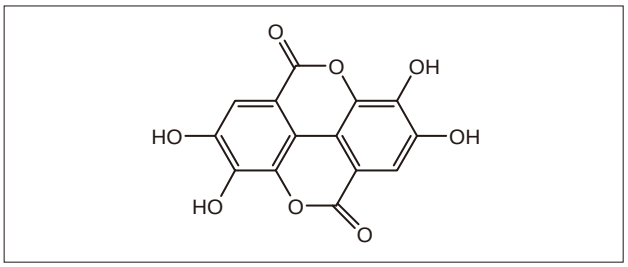


Fig. 1.22.4 Structure of Ellagic Acid

■Analytical Conditions

Column	: Shim-pack XR-Phenyl (50 mm L. × 3.0 mm I.D., 2.2 μm)
Mobile Phase	: A: 10 mmol/L Oxalic Acid aq. B: Methanol / Acetonitorile = 7/3 (v/v) B Conc. 35 %
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Volume	: 2 μL
Detection	: SPD-M20A (366 nm)
UV Cell	: Semi-micro cell

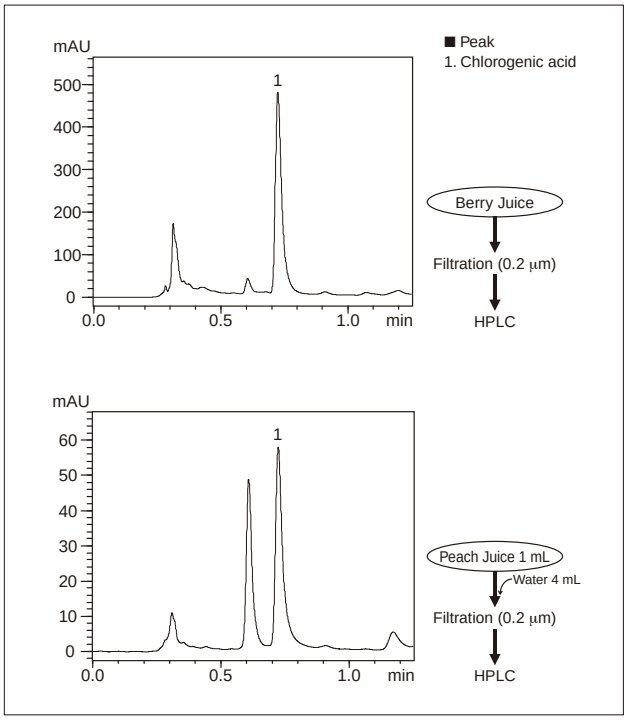


Fig. 1.22.3 Chromatograms of Berry Juice (upper) and Peach Juice (lower)

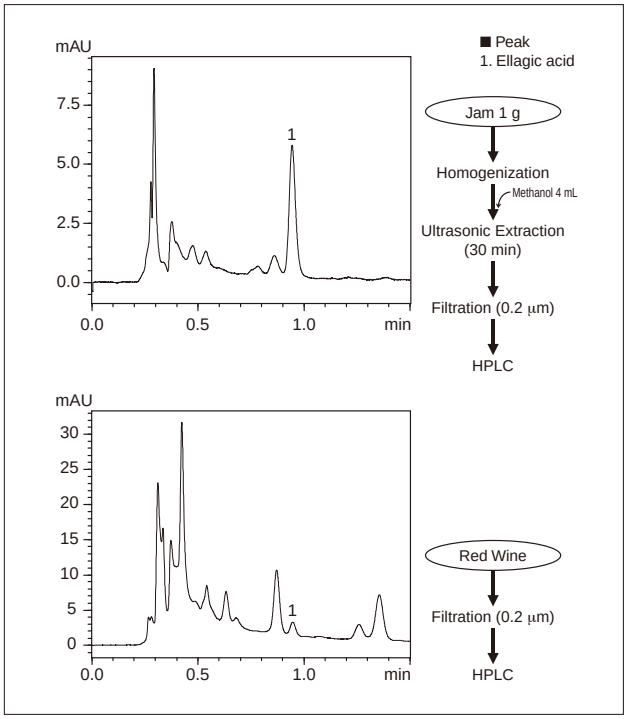


Fig. 1.22.5 Chromatograms of Raspberry Jam (upper) and Red Wine (lower)

1.23 High Speed Analysis of α -Acids and β -Acids in Hops (1) - LC

■Explanation

Hops are one of the basic ingredients in beer and contain α-acids (humulones) and β-acids (lupulones). During the brewing process the α-acids are converted through isomerization into iso-α-acids (isohumulons), which are largely responsible for the bitterness of beer. β-acids, on the other hand, do not produce much bitterness during the boiling stage of beer production, but they are said to play a role in "balancing" the bitter flavor during fermentation and storage.

HPLC is generally used for the analysis of α-acids and β-acids, but this analysis typically takes as long as 30 minutes. Here we introduce an example of high-speed analysis of α-acids and β-acids in hops using the Prominence UFLCXR ultra fast, high resolution LC system with a high-speed, high-resolution column.

■Analysis of Standard Solution

We selected 6 target analytes in hops, consisting of 3 α-acids (humulone, cohumulone, and adhumulone) and 3 β-acids (lupulone, colupulone, and adlupulone). The structural formulas of these substances are shown in Fig. 1.23.1. A standard solution was prepared by dissolving 0.1 g of the standard extract "International Calibration Extract 2" (American Society of Brewing Chemists)* in methanol, after which the volume was adjusted to 100 mL, and the resultant solution was filtered through a 0.22 µm membrane filter. Fig. 1.23.2 shows the results of measurement of a 4 µL injection of this standard solution. For the high-speed, high-resolution column, we used the Advanced Materials Technology HALO® C18 column (particle size 2.7 µm). The system pressure in this analysis was about 56 MPa at maximum.

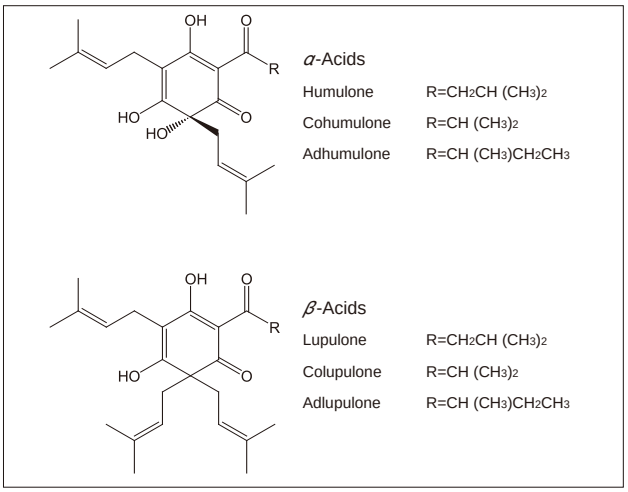


Fig. 1.23.1 Structures of α-Acids and β-Acids

■Analytical Conditions

Column	: HALO® C18 (150 mm L. × 3.0 mm I.D., 2.7 µm)
Mobile Phase	: A: Water/Methanol/Phosphoric Acid (85 %)/ Triethylamine = 300 mL/700 mL/19.6 g/15.1 g
	B: Methanol
	Gradient Elution Method
Time Program	: B 0 % (0 min) → 35 % (10 min)
Flowrate	: 1.1 mL/min
Column Temp.	: 50 °C
Injection Volume	: 4 µL
Detection	: SPD-20AV at 330 nm
Flow Cell	: Semi-micro cell

* Composition of "International Calibration Extract 2"

- Cohumulone	14.45 %
- Humulone + Adhumulone	34.94 %
- Colupulone	12.92 %
- Lupulone + Adlupulone	12.02 %

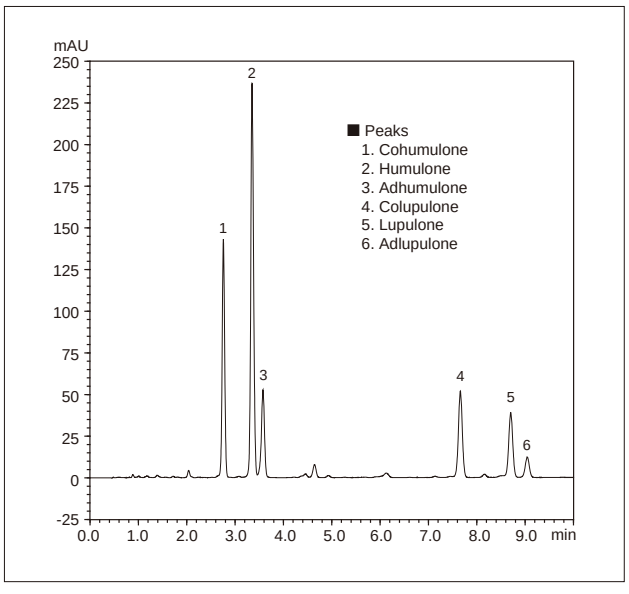


Fig. 1.23.2 Chromatogram of a Standard Mixture of α-Acids and β-Acid

1.23 High Speed Analysis of α -Acids and β -Acids in Hops (2) - LC

■Analysis of Hop Pellets

Fig. 1.23.3 shows the results of analysis of a commercially available hop pellets. Sample preparation was conducted according to the procedure[1] of Fig. 1.23.4. Even in the case of an actual sample, excellent separation was obtained just as with the standard solution (Fig. 1.23.2).

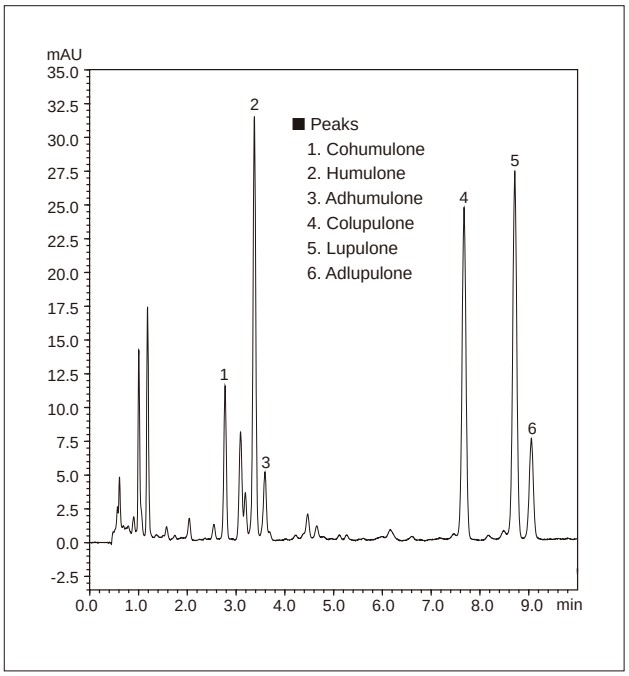


Fig. 1.23.3 Chromatogram of Hop Pellets

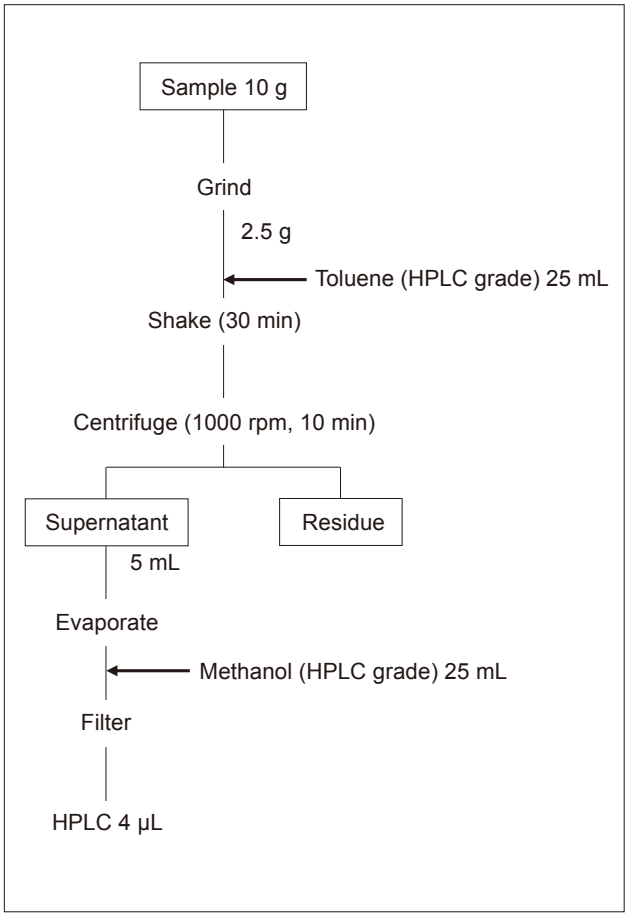


Fig. 1.23.4 Sample Preparation

[Reference]
1) Brewery Convention of Japan [Analysis Committee] Edition, Methods of Analysis of BCOJ (Revised edition), Brewing Society of Japan (2004)

1.24 High Speed Analysis of Iso-α-Acids and α-Acids in Beer (1) - LC

■Explanation

Iso-α-acids (iso-humulones) are compounds that contribute to the bitter taste of beers and are generated through the isomerization of α-acids (humulones) during the brewing process. Here we introduce an example of analysis of iso-α-acids and α-acids using the ultra-high speed, high resolution Prominence UFLCXR system with a high-speed, highresolution column.

■Analysis of Standard Solution

We selected 6 target analytes, consisting of 3 iso-α-acids (isohumulone, isocohumulone, and isoadhumulone, all *trans* isomers) and 3 α-acids (humulone, cohumulone, and adhumulone). The structural formulas of the iso-α-acids (*cis* and *trans* isomers) and α-acids are shown in Fig. 1.24.1. A standard solution was prepared by dissolving 0.2 g of the standard extract "International Calibration Standard I2" and "International Calibration Extract 2" (both American Society of Brewing Chemists)* in 50 mL of methanol. A 1/100 dilution of the stock solution (using methanol) was filtered through a 0.22 µm membrane filter. Fig. 1.24.2 shows the results of measurement of a 4 µL injection of this standard solution. For the high-speed, high-resolution column, we used the AMT HALO® C18 column (particle size 2.7 µm). The maximum system pressure during analysis was approximately 56 MPa (8100 psi).

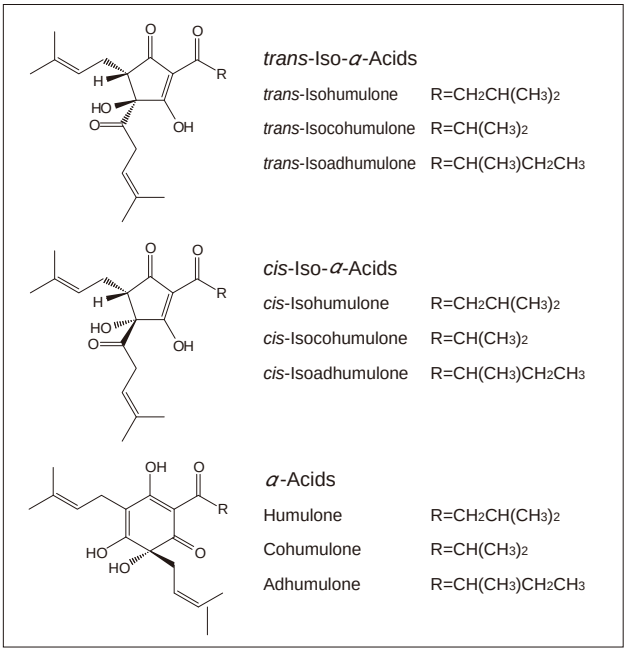


Fig. 1.24.1 Structures of Iso-α-Acids and α-Acids

■Analytical Conditions

Column	: HALO® C18 (150 mm L. × 3.0 mm I.D., 2.7 µm)
Mobile Phase	: 0.1 % Phosphoric Acid (85 %), 0.2 mmol/L EDTA·2Na aq. / Acetonitrile = 45/55 (v/v)
Flowrate	: 1.4 mL/min
Column Temp.	: 30 °C
Injection Volume	: 4 µL
Detection	: SPD-20AV at 270 nm (0-7.2 min), 330 nm (7.2-13.0 min)
Flow Cell	: Semi-micro cell

* Composition of "International Calibration Standard I2"
Isohumulone + isocohumulone + isoadhumulone: 64.3 % (all *trans* isomers)

* Composition of "International Calibration Extract 2"
- Cohumulone: 14.45 %
- Humulone + Adhumulone: 34.94 %
- Colupulone: 12.92 %
- Lupulone + Adlupulone: 12.02 %

*** Since the β-acids (colupulone, lupulone, and adlupulone) all elute after 13 minutes under these analytical conditions and were not of interest in this note, an acetonitrile rinse step was added when conducting analysis of the standard solution to flush the β-acids from the column.

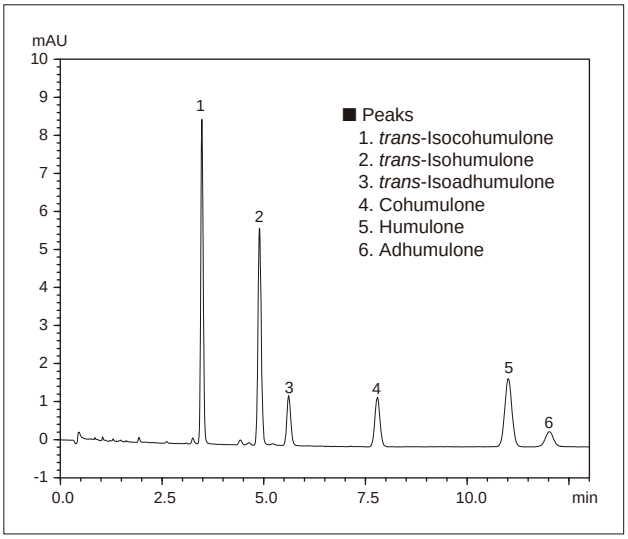


Fig. 1.24.2 Chromatogram of Standard Mixture of Iso-α-Acids and α-Acids

1.24 High Speed Analysis of Iso-α-Acids and α-Acids in Beer (2) - LC

■Analysis of Beer

Fig. 1.24.3 shows the result of analysis of a commercial beer. Sample preparation was conducted according to the procedure[1] shown in Fig. 1.24.4. Peaks a, b, and c in the chromatogram are presumed[2] to be the *cis* isomers of isocohumulone, isohumulone, and isoadhumulone, respectively.

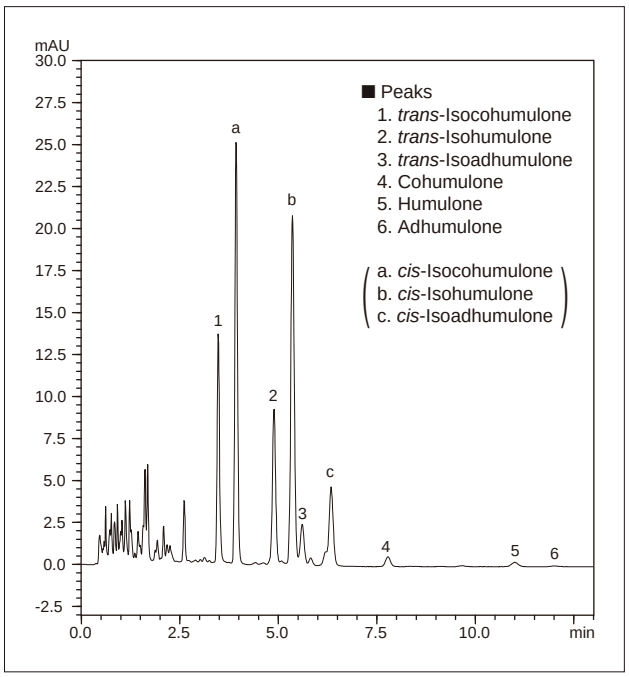


Fig. 1.24.3 Chromatogram of Beer Sample

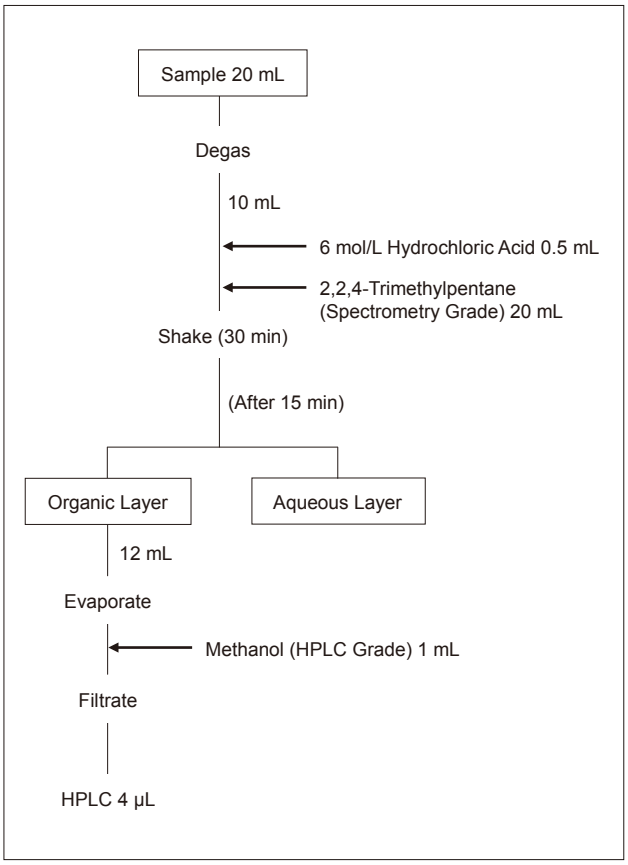


Fig. 1.24.4 Sample Preparation

[References]

1) Brewery Convention of Japan [Analysis Committee] Edition, Revised BCOJ Beer Analysis Method, Brewing Society of Japan (2004)

2) B. Jaskula, K. Goiris1, G. De Rouck, G. Aerts, L. De Cooman : J. Inst. Brew., 113(4), 381-390 (2007)(see pdf)

1.25 Analysis of Lycopene and β-Carotene in Tomato - LC

■Explanation

Lycopene is a type of carotenoid, and is found in large quantities as a red pigment in red tomatoes, etc. The antioxidative effects of lycopene are said to be 100 times stronger than that of vitamin E and more than twice stronger than that of β-carotene, and lycopene is receiving attention for its effects to prevent lifestyle diseases such as cancer and arteriosclerosis, and to slow aging. Though lycopene and β-carotene have similar structures, they can be easily separated by reversed phase chromatography using a non-aqueous mobile phase.

■Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D.)
Mobile Phase	: Acetonitrile / Ethanol = 4/1 (v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 50 °C
Injection Volume	: 5 µL
Detection	: SPD-M20A 450 nm Slit width: 8 nm, Band width: 8 nm
Cell Temp.	: 50 °C

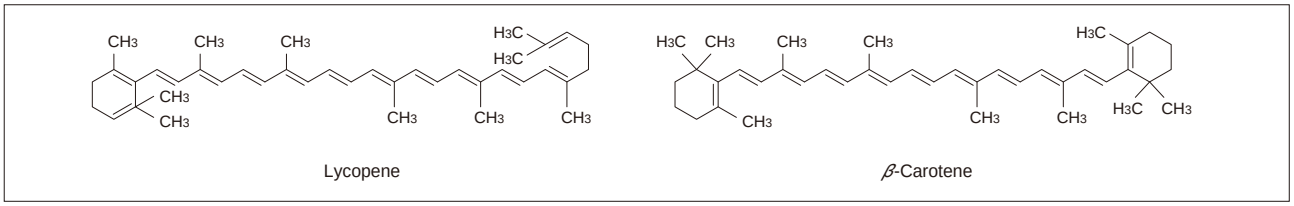


Fig. 1.25.1 Structure of Lycopene and β-Carotene

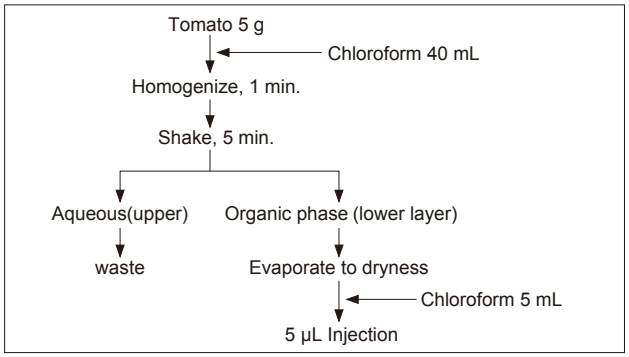


Fig. 1.25.2 Sample Preparation

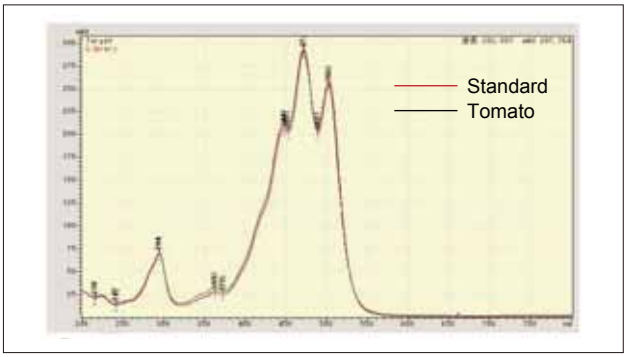


Fig. 1.25.4 Spectra of Lycopene

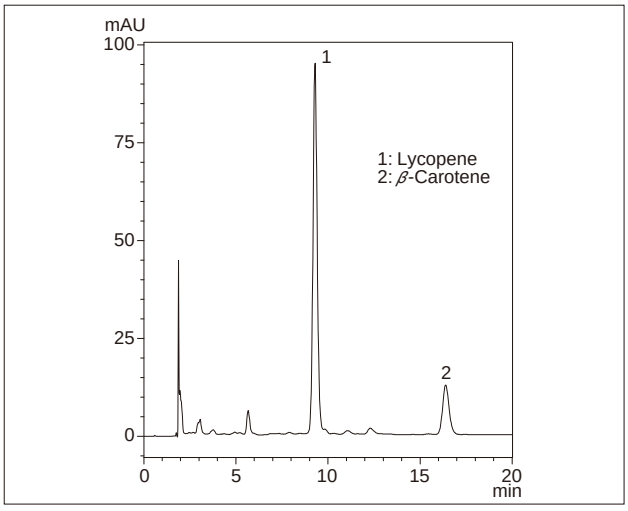


Fig. 1.25.3 Chromatogram of Tomato

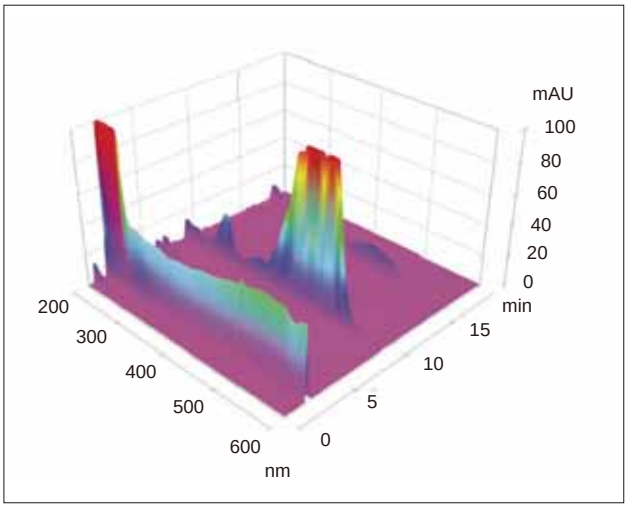


Fig. 1.25.5 3-D Chromatogram of Tomato

1.26 Determination of Cyanidin-3-Glucoside in Black Soybeans - LC

■Explanation

Anthocyanins, essential factor to fix the color tone of flowers and fruits, are the generic name of the glycosides which have anthocyanidins as aglycones. The color tone of anthocyanins varies greatly according to the pH. It is known that many types of anthocyanins exist widely throughout higher plants, and recently they are receiving much attention due to their antioxidative properties. Among these, cyanidin-3-glucoside, the glucose glycoside of cyanidin, is said to account for more than 90 % of the anthocyanidins present in black soybeans. Here we introduce an analysis of cyanidin-3-glucoside using the Prominence SPD-M20A photodiode array detector.

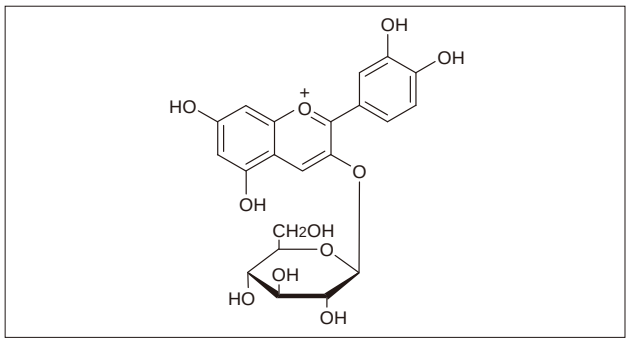


Fig. 1.26.1 Structure of Cyanidin-3-Glucoside

■Analysis of Black Soybeans

Fig. 1.26.2 shows the chromatogram of commercial black soybean extract solution obtained at two wavelengths. After pulverizing the black soybeans, 5 mL of methanol containing 1 % hydrochloric acid was added to 1 g of the ground powder. Cyanidin-3-Glucoside was extracted using ultrasonication, and centrifugation and filtering to the extract were performed. And then, mobile phase A was added to make a 10-fold dilution. Fig. 1.26.3 shows the spectra of the cyanidin-3-glucoside in the black soybean extract solution and the standard solution. A 3-D plot is shown in Fig. 1.26.4.

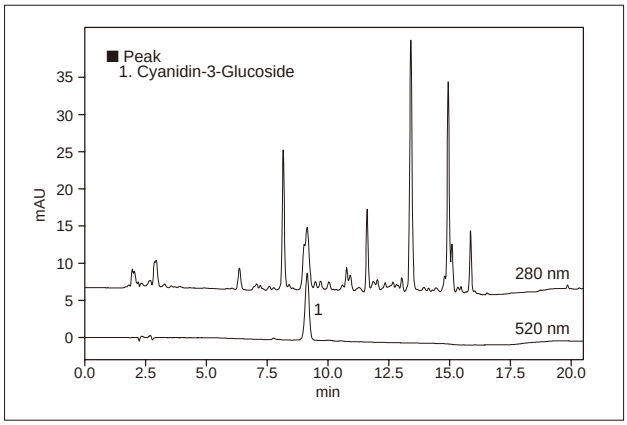


Fig. 1.26.2 Chromatograms of Black Soybean Extract (10 µL Inj.)

■Analytical Conditions

Column : Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D.)
Guard Column Shim-pack GVP-ODS (10 mm L. × 4.6 mm I.D.)
Mobile Phase : A: 10 mmol/L (Sodium) Phosphate Buffer (pH 2.6)
B: Acetonitrile
Initial B.Conc = 5 %

Time (min)	B.Conc (%)
10.00	30
15.00	100
20.00	100
20.01	5
35.00	STOP

Flowrate : 1.0 mL/min
Column Temp. : 40 °C
Detection : SPD-M20A at 520 nm (Slit width: 8 nm)

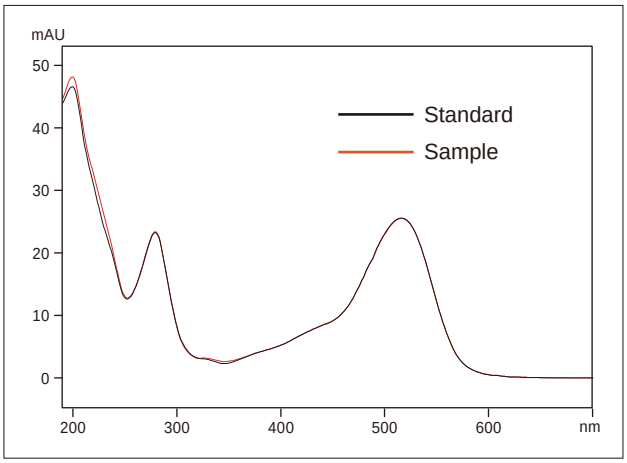


Fig. 1.26.3 Spectra of Cyanidin-3-Glucoside in Black SoybeanExtract and Standard Compound

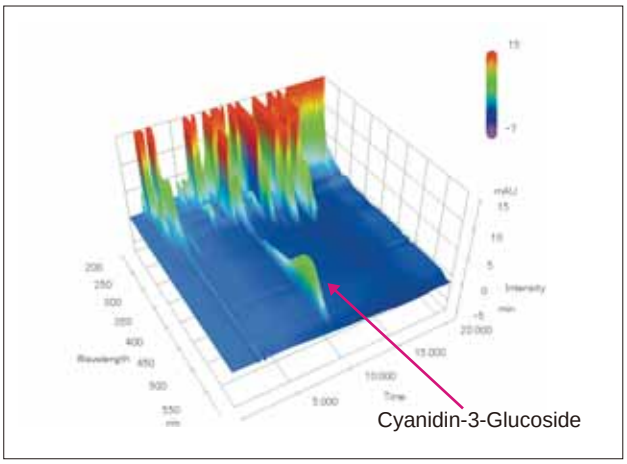


Fig. 1.26.4 3-D Plot of Black Soybean Extract

1.27 Analysis of Capsaicinoids in Spices - LC

■Explanation

When absorbed by the body, capsaicins, the spice element found in chili pepper, has the effect of stimulating adrenaline secretion and causing perspiration. Since ancient times, capsaicins have been known for their anti-bacterial, stomachic, and body-warming properties. Furthermore, capsaicins are very stable elements that maintain their spiciness even when used in a variety of cooking methods. The unit used to indicate the spiciness of a spice is known as the "Scoville Scale". This scale is indicated in magnitudes based on the amount of time until a taster no longer feels the spiciness of a spice extract dissolved in sugar water. In recent years, to gain more objective indicators, quantitative methods that use HPLC to analyze the capsaicinoids in a spice also are in use. This issue introduces examples of the analysis of capsaicinoids in spice using HPLC.

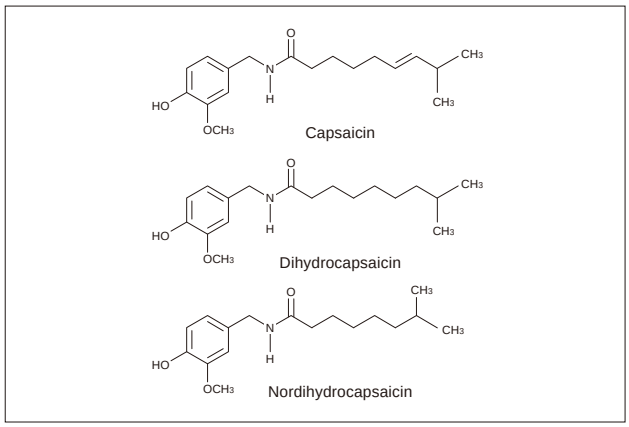


Fig. 1.27.1 Structure of Capsaicinoids

■Analysis of Spice

Fig. 1.27.2 shows the sample preparation steps used on the commercial spices and Figs. 1.27.3, Figs. 1.27.4 show the results of the analysis. With the actual samples, because impurity substances are eluted out after the dihydrocapsaicin, in order to remove those substances from the column we recommend cleansing the column by flowing the mobile phase with increased level of acetonitrile before each analysis.

* The peak indicated by the arrow in the chromatogram is assumed to be nordihydrocapsaicin.

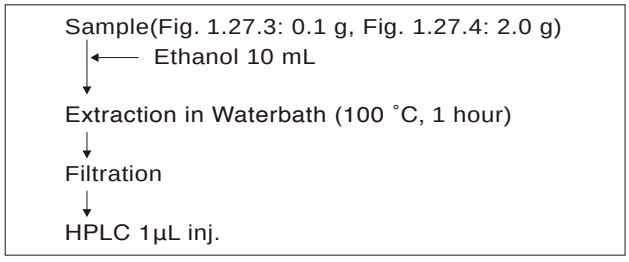


Fig. 1.27.2 Sample Preparation

■Analytical Conditions

Column	: Shim-pack VP-ODS (150 mL. × 4.6 mm I.D.)
Mobile Phase	: 1.0 % Acetic Acid aq. / Acetonitrile = 1/1(v/v)
Flowrate	: 1.2 mL/min
Column Temp.	: 40 °C
Injection Volume	: 1 µL
Detection	: SPD-20A at 280 nm
	RF-10AXL Ex at 280 nm, Em at 325 nm

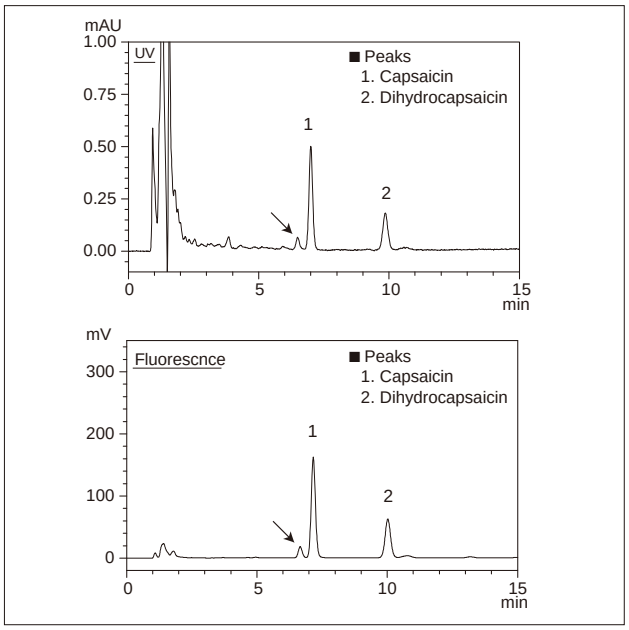


Fig. 1.27.3 Chromatogram of Red Pepper

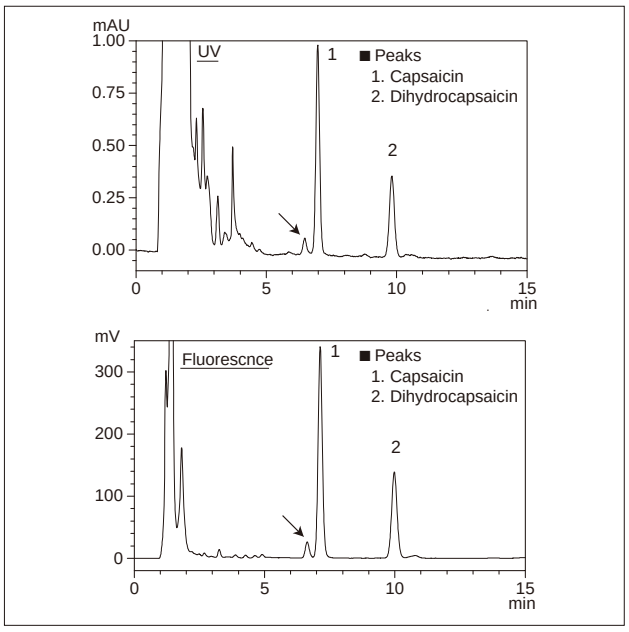


Fig. 1.27.4 Chromatogram of Pepper Sauce

1.28 High Speed Analysis of Isothiocyanates and Sinigrin - LC

■Explanation

The isothiocyanates contained in cruciferous vegetables, such as Japanese horseradish and mustard, have been receiving attention for their antibacterial and antioxidative properties. It is known that these pungent components usually exist as glycosides in plant tissue, and when they are hydrolyzed by myrosinase, they take part in a transfer reaction, and are released as isothiocyanates.
An example of the simultaneous analysis of the isothiocyanates in Japanese horseradish and sinigrin, a representative glycoside, performed using the Prominence UFLC ultra fast LC system and the Shim-pack XR-ODS high-performance column, which is used in high-speed, high-resolution applications, is presented here.

■Simultaneous Determination of Isothiocyanates and Sinigrin in Japanese Horseradish

The simultaneous analysis of isothiocyanates and the representative glycoside sinigrin (Fig. 1.28.1) in powdered Japanese horseradish was performed using a Shim-pack XR-ODS column (50 mm) under high-speed separation conditions. Fig. 1.28.2 shows an example of the analysis of standard solution. When water is added to the sinigrin in Japanese horseradish, hydrolysis occurs, and mainly allyl isothiocyanate is released. On comparing the results obtained immediately after adding water to commercially available dried, powdered horseradish and those obtained after adding water, kneading the mixture, and waiting 5 minutes (Fig. 1.28.3-upper chromatogram: immediately after; lower chromatogram: after waiting 5 minutes), there are certain changes in peak intensities of sinigrin and allyl isothiocyanate.

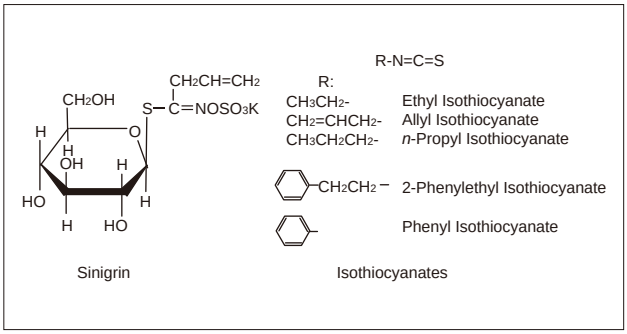


Fig. 1.28.1 Structures of Isothiocyanates and Sinigrin

■Analytical Conditions

Column	: Shim-pack XR-ODS (50 mm L. x 3.0 mm I.D., 2.2 µm)
Mobile Phase	: A: 10 mmol /L (Imidazole) Phosphate Buffer [pH 2.6] B: Acetonitrile Gradient Elution Method
Time Program	: B 0 % (0-1.5 mim) → 50 % (1.5-2.0 mim) → 50 % (2.0-7.0 mim) → 0 % (7.0-9.0 mim)
Flowrate	: 1.2 mL/min
Column Temp.	: 40 °C
Injection Volume	: 10 µL
Detection	: SPD-M20A at 230 nm (0-2.4 min) and 240 nm (after 2.4 min)
Flow Cell	: Semi-micro cell

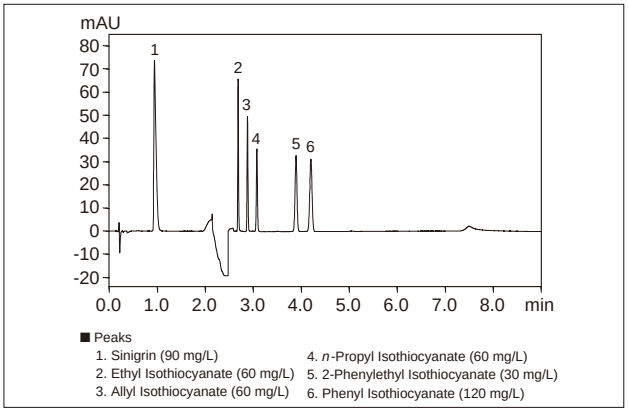


Fig. 1.28.2 Chromatogram of a Standard Mixture of Isothiocyanates and Sinigrin

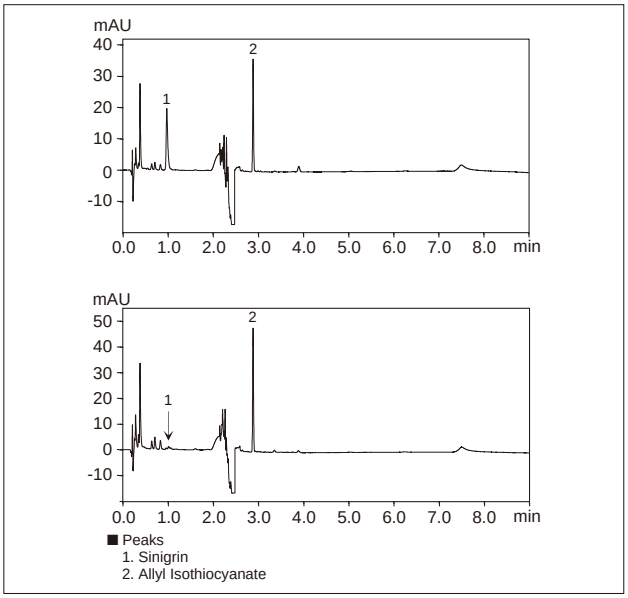


Fig. 1.28.3 Chromatogram of Jpanese Horseradish
Upper: Immediately after adding water to the sample
Lower: 5 minutes after adding water to the sample

1.29 High Speed Analysis of Isoflavones in Soy - LC

■Explanation

Soy isoflavones are a group of compounds that are present in large quantity in soybeans, and especially in the soy germ. Recently, these compounds are receiving much attention due to the similarity of their activity with the female hormone estrogen. Here we introduce an example of the simultaneous analysis of seven isoflavones using the high-performance "Shim-pack XR-ODS" column. Soy food products (soybean flour, miso, soymilk) were prepared according to the procedure shown in Fig. 1.29.2, and high-speed analysis was conducted as shown in Fig. 1.29.3 through 5.

■Analytical Conditions

Column	: Shim-pack XR-ODS (50 mm L. x 3.0 mm I.D., 2.2 µm)
Mobile Phase	: A : 0.1 % Formic Acid -Water B : Acetonitrile Gradient Elution Method
Time Program	: B 15 % (0 min) → 23 % (2 min) →75 % (2.3-2.5 min) → 15 % (2.51-4 min)
Flowrate	: 1.5 mL/min
Column Temp.	: 40 °C
Injection Volume	: 4 µL
Detection	: SPD-20A at 254 nm
Flow Cell	: Semi-micro cell

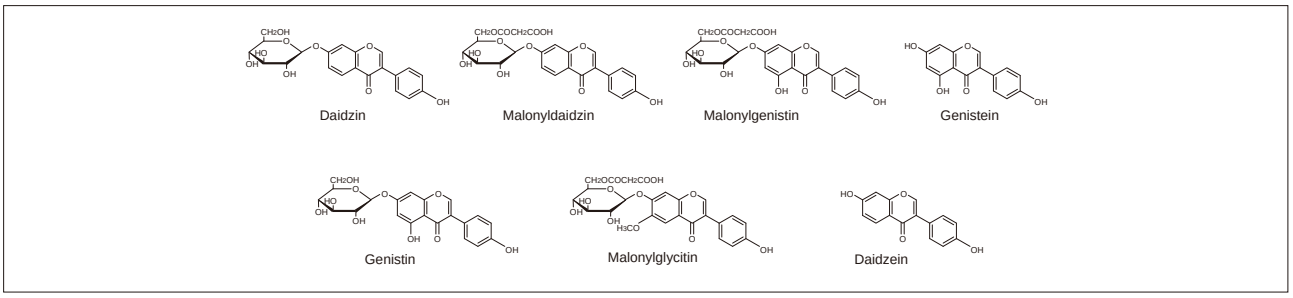


Fig. 1.29.1 Structural Formulas of Seven Isoflavones

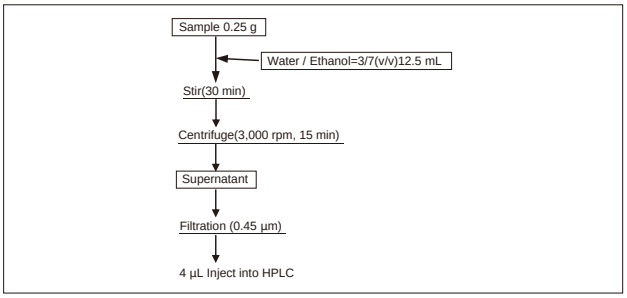


Fig. 1.29.2 Sample Preparation

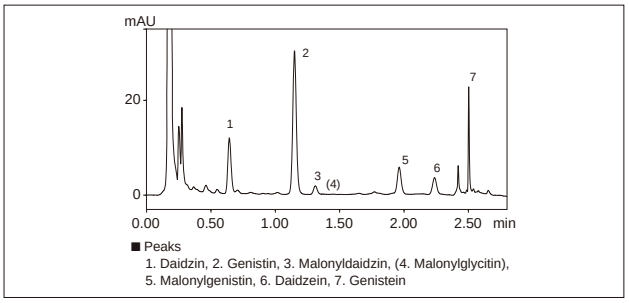


Fig. 1.29.4 Chromatogram of Miso (Soybean Paste)

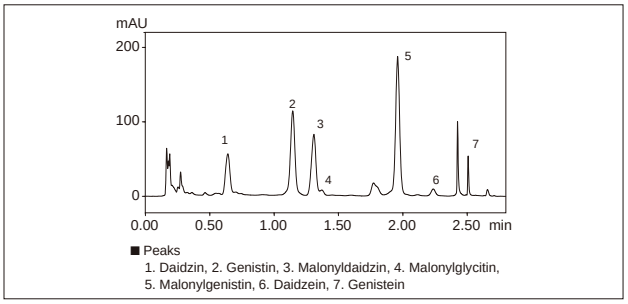


Fig. 1.29.3 Chromatogram of Soybean Flour

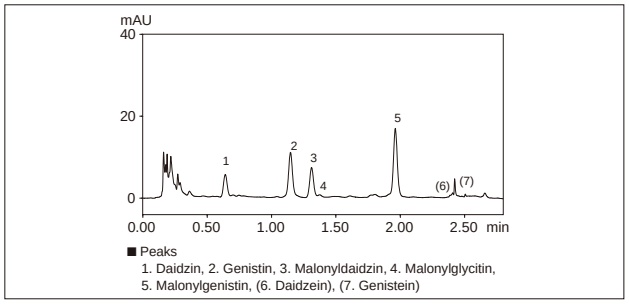


Fig. 1.29.5 Chromatogram of Soymilk

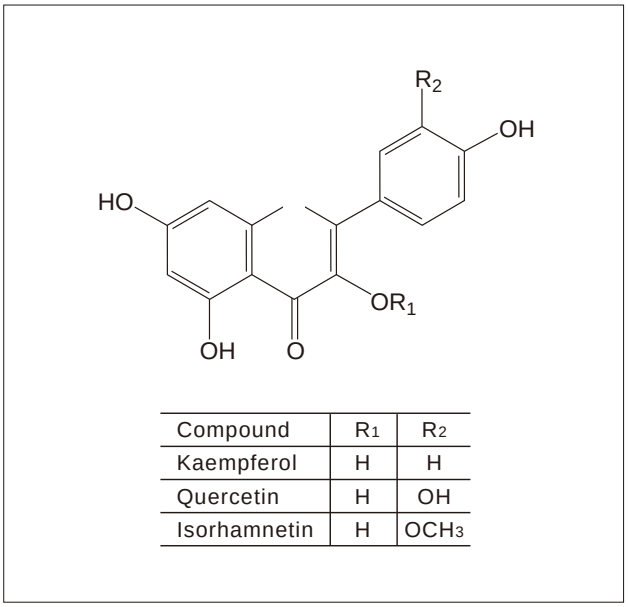
1.30 Analysis of Flavonoids in Ginkgo Biloba Extract (1) - LC

■Explanation

Flavonoids are a kind of polyphenol, and the name also refers to a class of plant metabolites. Recently, there has been much research on flavonoids with regard to their physiological activity, and in particular, their anti-oxidative effects have been reported. Ginkgo biloba leaves are said to contain as many as 20 types of flavonoids, and among these are quercetin, kaempferol and isorhamnetin, 3 types that are present in large quantities. Here we introduce an example of the analysis of these 3 flavonoids present in ginkgo biloba leaves using the SPD-M20A photodiode array detector.

■Analysis of Standard Solution

Fig. 1.30.1 shows the structures of the 3 flavonoids (quercetin, kaempferol and isorhamnetin) analyzed here. Fig. 1.30.2 shows the UV-VIS absorption spectrum of quercetin, indicating that all of these compounds display maximum absorption in the vicinities of 250-260 nm and 370 nm. Fig. 1.30.3 shows a chromatogram of the 3 flavonoids in a standard solution at 370 nm. For detection, the SPD-M20A photodiode array detector was used.



Compound	R_1	R_2
Kaempferol	H	H
Quercetin	H	OH
Isorhamnetin	H	OCH_3

Fig. 1.30.1 Structures of 3 Flavonoids

■Analytical Conditions

Column	: Shim-pack VP-ODS (250 mm L. x 4.6 mm I.D.)
Mobile Phase	: A: 1.0 % Phosphoric Acid (85 %) aq. B: Acetonitrile Gradient Elution Method
Time Program	: B 30 % (0-12 min) → 90 % (12.01-15 min) → 30 % (15.01-20 min)
Flowrate	: 1.5 mL/min
Column Temp.	: 60 °C
Injection Volume	: 10 µL
Detection	: SPD-M20A at 370 nm

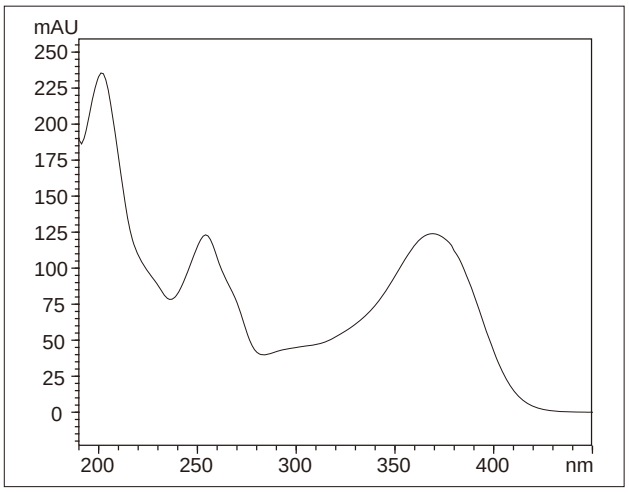


Fig. 1.30.2 UV-VIS Spectrum of Quercetin

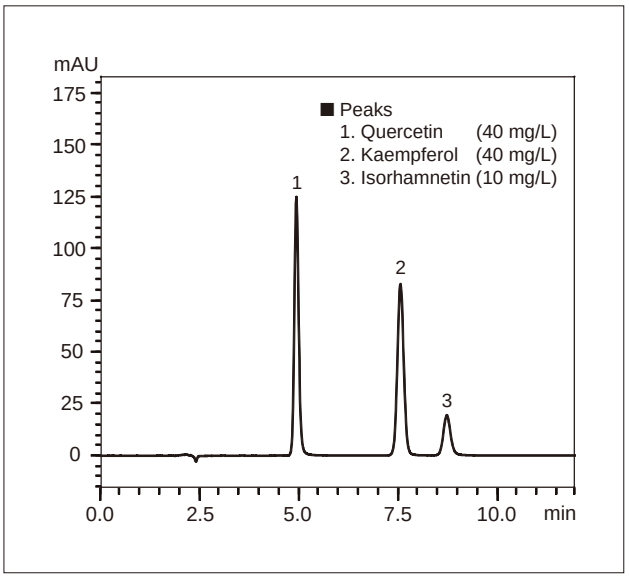


Fig. 1.30.3 Chromatogram of a Standard Mixture of 3 Flavonoids

1.30 Analysis of Flavonoids in Ginkgo Biloba Extract (2) - LC

■Analysis of Ginkgo Biloba Extract Supplement

We conducted analysis of a commercially available ginkgo biloba leaf extract (tablet) after preparing the sample according to the procedure shown in Fig. 1.30.4. Fig. 1.30.5 shows the obtained chromatogram. Fig. 1.30.6 shows the respective spectra of the flavonoids in the sample overlaid with the corresponding standard spectra.

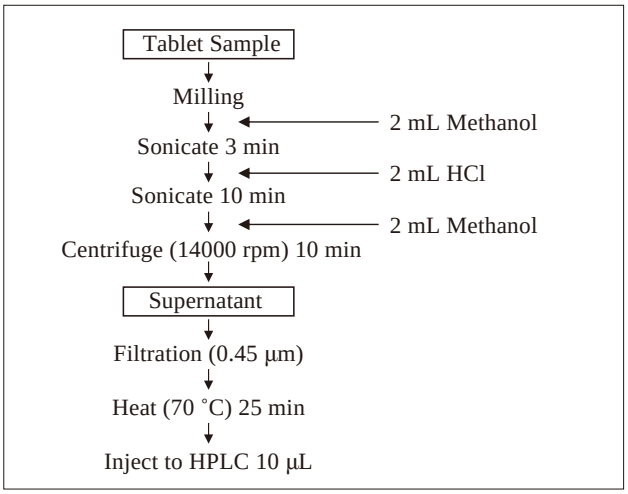


Fig. 1.30.4 Sample Preparation

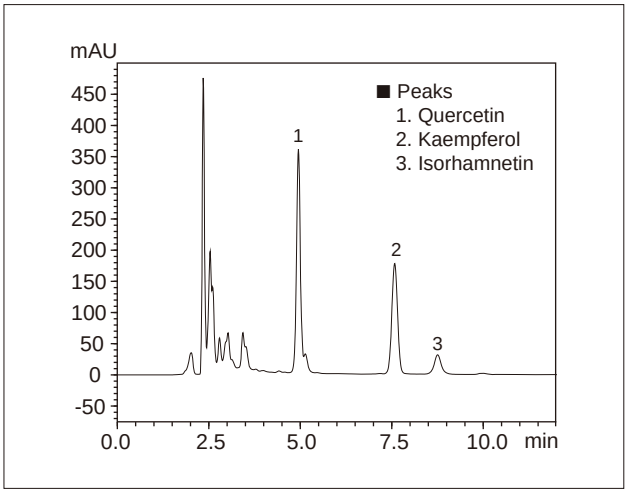


Fig. 1.30.5 Chromatogram of Ginkgo Biloba Dietary Supplement

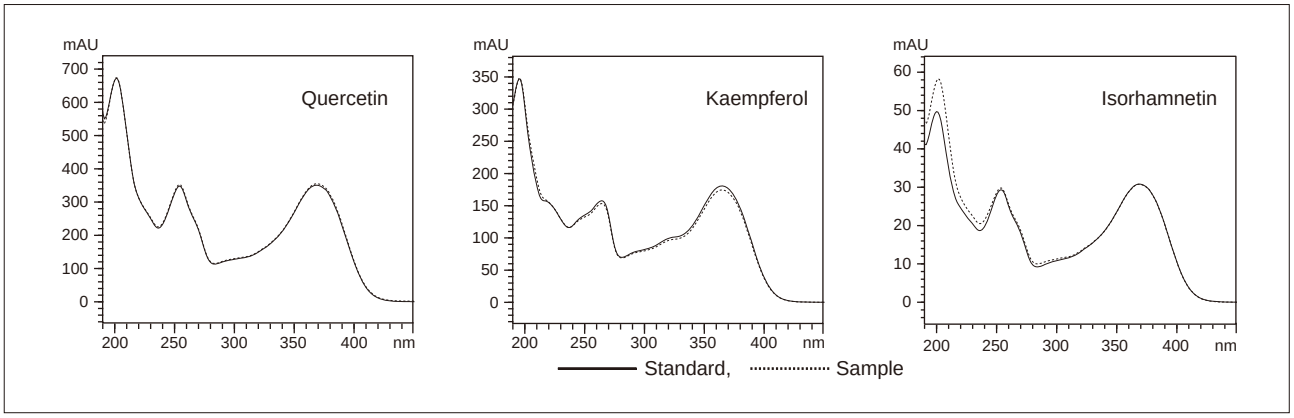


Fig. 1.30.6 UV-VIS Spectra of 3 Flavonoids in Ginkgo Biloba Dietary Supplement

[Reference]
United States Pharmacopeia (USP32-NF27)

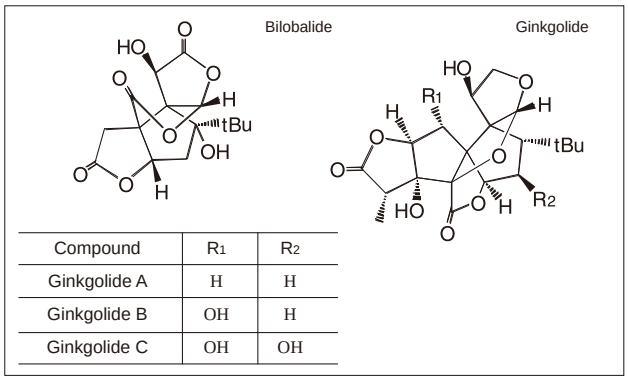
1.31 Analysis of Terpenoids in Ginkgo Biloba - LC

■Explanation

Ginkgo biloba extract contains flavonoids and terpenoids that have been reported to be effective for improving poor blood circulation in the brain as well as poor peripheral blood vessel circulation. This ginkgo biloba extract is used as a health dietary supplement in Japan and the United States. Here we present an example of analysis of terpenoids in ginkgo biloba extract using the ELSD-LT Ⅱ evaporative light scattering detector.

■Analysis of Standard Solution

Terpenoids that are known to be present in large quantities in ginkgo biloba include bilobalide, ginkgolide A, ginkgolide B and ginkgolide C (Fig. 1.31.1). Because these compounds have no chromophores, use of the evaporative light scattering detector together with reversed-phase gradient elution is an effective means of analysis. Fig. 1.31.2 shows a chromatogram obtained from analysis of a standard solution of these four terpenoids (200 mg/L each, methanol).



Compound	R_1	R_2
Ginkgolide A	H	H
Ginkgolide B	OH	H
Ginkgolide C	OH	OH

Fig. 1.31.1 Structures of 4 Terpenoids

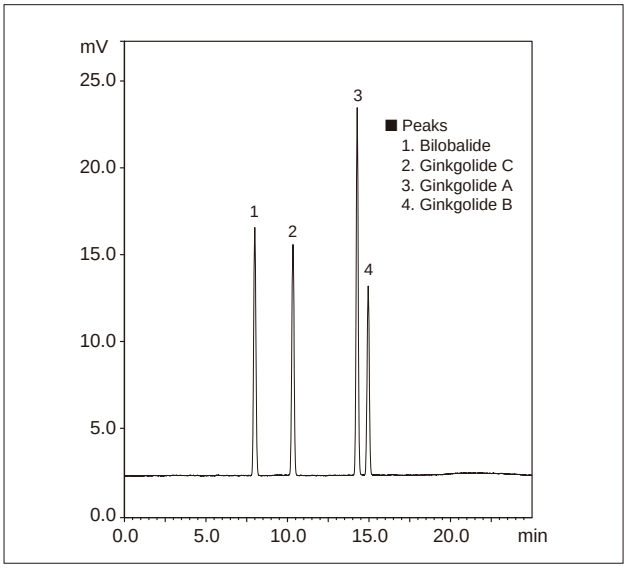


Fig. 1.31.2 Chromatogram of a Standard Mixture of 4 Terpenoids in Ginkgo Biloba (200 mg/L each, 10 µL injected)

■Analytical Conditions

Column : Shim-pack FC-ODS (150 mm L. × 4.6 mm I.D.)
Mobile Phase : A: Water
B: Methanol
Gradient Elution Method
Time Program : B 20 % (0 min) → 45 % (16 min) → 80 % (16.01-20 min) → 20 % (20.01-30 min)
Flowrate : 1.0 mL/min
Column Temp. : 50 °C
Injection Volume : 10 µL
Detection : ELSD-LT Ⅱ
Temperature : 40 °C
Gain : 6
Nebulizer Gas : N_2
Gas Pressure : 350 kPa

■Analysis of Dietary Ginkgo Biloba Supplement

Analysis was conducted after performing sample preparation of a commercially available dietary ginkgo biloba supplement according to the procedure shown in Fig. 1.31.3. Fig. 1.31.4 shows the chromatogram.

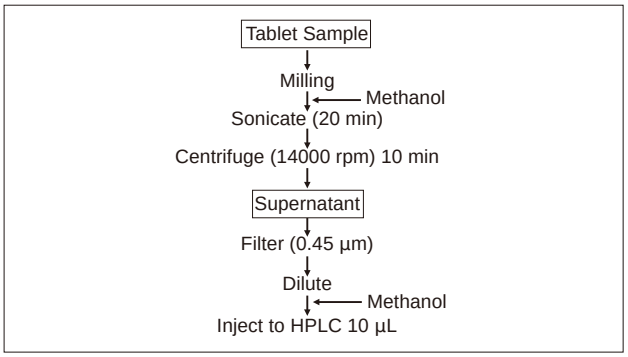


Fig. 1.31.3 Sample Preparation

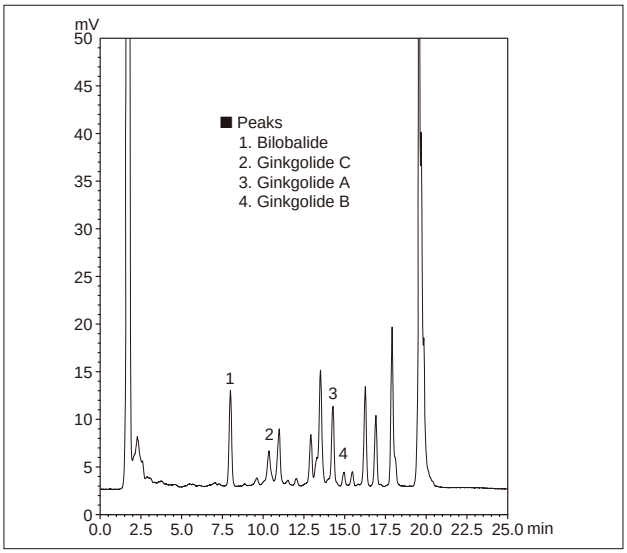


Fig. 1.31.4 Chromatogram of Dietary Ginkgo Biloba Supplement

1.32 Analysis of Ginkgolic Acids in Ginkgo Biloba Extract (1) - LC

■Explanation

Ginkgo leaf extract (ginkgo biloba extract), which contains active ingredients extracted from ginkgo biloba leaves, is reported to be effective in improving cerebral and peripheral blood circulation deficiencies.
It is used in Japan and the United States in the form of a dietary supplement, and in Germany, France, and other European countries as a prescription medication. However, alkylphenols which are present in the ginkgolic acids contained in ginkgo leaves are known to cause allergic reactions. For this reason, the United States Pharmacopeia (USP) has established an upper limit for ginkgolic acid content in ginkgo biloba extract. Here we introduce an example of analysis of gingkolic acids contained in ginkgo biloba leaves.

■Analysis of Standard Solution

The ginkgolic acids in ginkgo biloba leaves that were analyzed include ginkgolic acid C13:0 (hereafter, GA C13:0), GA C15:0, GA C15:1, and GA C17:1. Fig. 1.32.1 shows the structural formula of these 4 substances. Due to the high hydrophobicity of these ginkgolic acids, the Shim-pack CLC-C8 in which the silica gel is modified with an octyl group (C8) was used, and chromatography was conducted using gradient elution. For detection, the SPD-M20A photodiode array detector was used. Fig. 1.32.2 shows the spectrum of GA C17:1, and Fig. 1.32.3 shows a chromatogram of a standard mixture of 4 ginkgolic acids.

■Analytical Conditions

Column	: Shim-pack CLC-C8 (250 mm L. x 4.6 mm I.D.)
Mobile Phase	: A: 0.01 % Phosphoric Acid (85 %) - Water B: 0.01 % Phosphoric Acid (85 %) - Acetonitrile Gradient Elution Method
Time Program	: B 80 % (0 min) → 90 % (15-18 min) → 80 % (18.01-25 min)
Flowrate	: 1.0 mL/min
Column Temp.	: 35 °C
Injection Volume	: 20 µL
Detection	: SPD-M20A at 311 nm

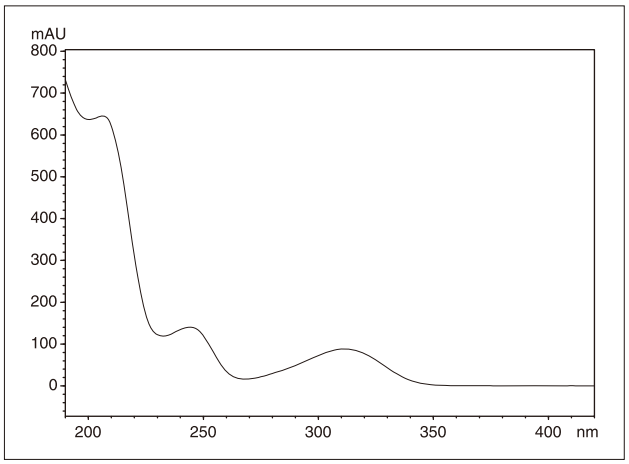
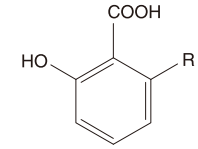


Fig. 1.32.2 UV Spectrum of Ginkgolic Acid C17:1



Compound		R
GA C13:0	Ginkgolic Acid C13:0	$C_{13}H_{27}$
GA C15:0	Ginkgolic Acid C15:0	$C_{15}H_{31}$
GA C15:1	Ginkgolic Acid C15:1	$C_{15}H_{29}$
GA C17:1	Ginkgolic Acid C17:1	$C_{17}H_{33}$

Fig. 1.32.1 Structures of Ginkgolic Acids

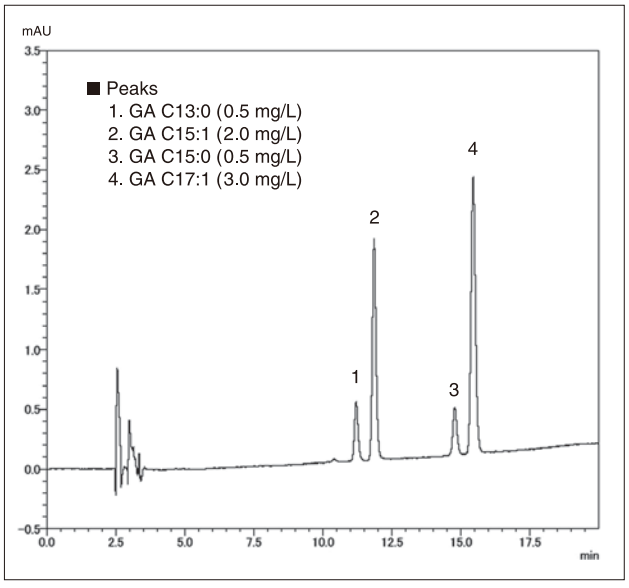


Fig. 1.32.3 Chromatogram of a Standard Mixture of 4 Ginkgolic Acids

1.32 Analysis of Ginkgolic Acids in Ginkgo Biloba Extract (2) - LC

■Analysis of Ginkgo Biloba Extract Supplement

Analysis of the ginkgo biloba extract was conducted after performing sample pretreatment of the commercial supplement containing the extract as shown in Fig. 1.32.4. Fig. 1.32.5 shows the chromatogram. Since ginkgolic acid was barely detected in this supplement, the chromatogram also shows the results of analysis of the prepared sample solution spiked with ginkgolic acid standard.

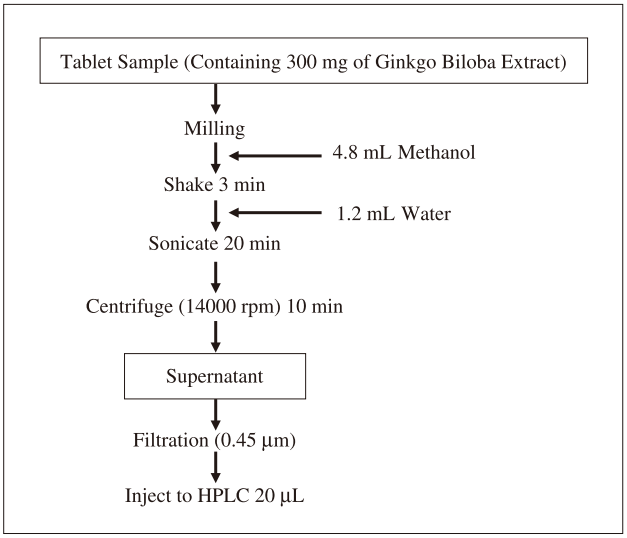


Fig. 1.32.4 Sample Preparation

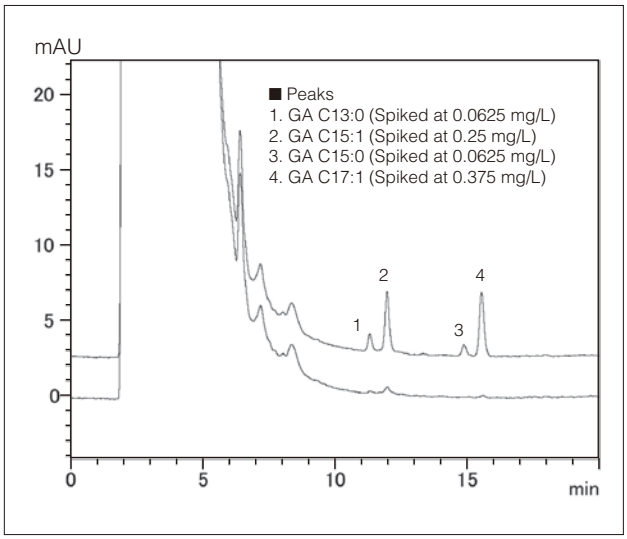


Fig. 1.32.5 Chromatogram of Ginkgo Biloba Extract Supplement (Upper: Spiked, Lower: Not Spiked)

[Reference]
United States Pharmacopeia (USP32-NF27)

1.33 High Speed Analysis of Gingerol and Shogaol in Ginger (1) - LC

■Explanation

Ginger is not only used as a spice, but has also been used in traditional Chinese herbal medicine since ancient times. Investigation into the efficacy of ginger has been attracting a lot of attention in recent years, and the use of ginger in health foods has seen great increases. Here we introduce an example of the analysis of 6-gingerol and 6-shogaol, constituents of ginger, using the Prominence UFLC ultra fast LC system with the SPD-M20A photodiode array detector.

■Analysis of Standard Solution

Fig. 1.33.1 shows the structures of 6-gingerol and 6-shogaol. Homologs of both gingerol and shogaol exist, and in the case of gingerol, these include 6-gingerol, 8-gingerol and 10-gingerol, with 6-gingerol the most abundant found in ginger. Gingerol is converted to shogaol during the dehydration reaction caused by heating, but the content levels of these constituents vary depending on the type of ginger. Fig. 1.33.2 shows the chromatogram obtained from analysis of a 6-gingerol and 6-shogaol standard solution (each 100 mg/L, in methanol solution) using the Shim-pack XR-ODS high-speed, high-resolution column. It should be noted that a column rinsing procedure was added to these conditions for analysis of the actual sample.

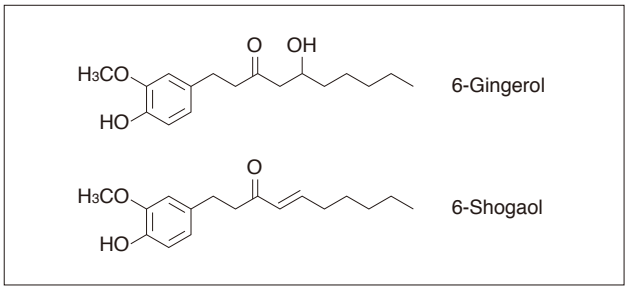


Fig. 1.33.1 Structures of 6-Gingerol and 6-Shogaol

■Analytical Conditions

Column	: Shim-pack XR-ODS (75 mm L. x 3.0 mm I.D. 2.2 µm)
Mobile Phase	: A: Water B: Acetonitrile Gradient Elution Method
Time Program	: B 30 → 90 % (0.00-2.10 min) → 90 % (2.50 min) → 100 % (2.51-3.50 min) → 30 % (3.51 min)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Volume	: 2 µL
Detection	: SPD-M20A at 280 nm (slit width: 8 nm)
Flow Cell	: Semi-micro cell

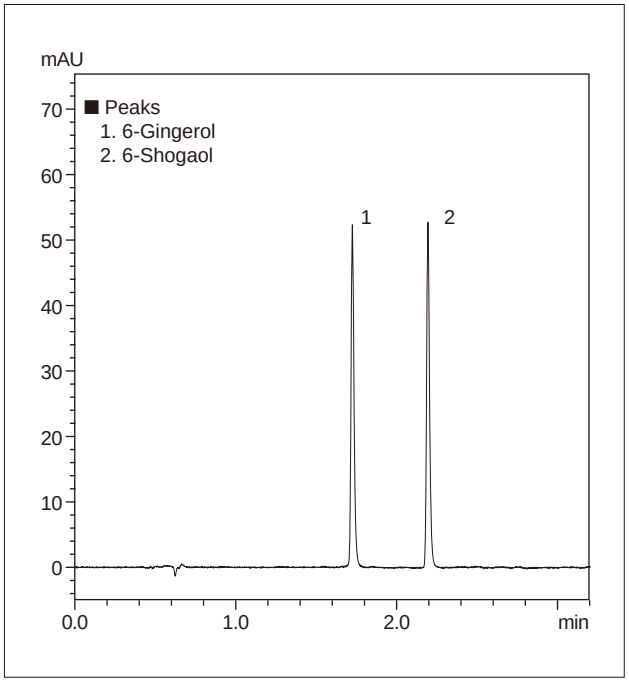


Fig. 1.33.2 Chromatogram of a Standard Mixture of 6-Gingerol and 6-Shogaol (100 mg/L, 2 µL injected)

1.33 High Speed Analysis of Gingerol and Shogaol in Ginger (2) - LC

■Analysis of Ginger Extract

After sample preparation according to the procedure of Fig. 1.33.3, analysis of ginger was conducted. Fig. 1.33.4 shows the resulting chromatogram. Fig. 1.33.5 shows the overlaid spectra of 6-gingerol obtained from analysis of a 6-gingerol standard sample and of a ginger extract.

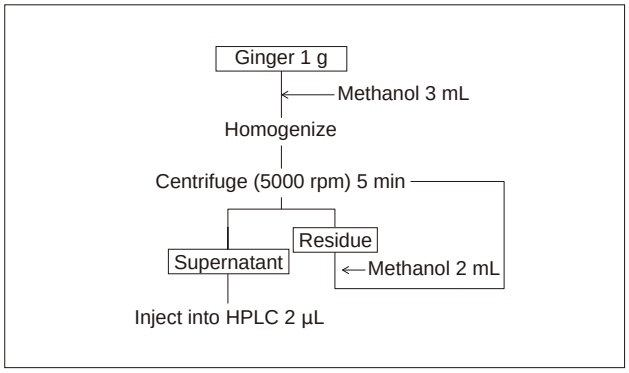


Fig. 1.33.3 Sample Preparation

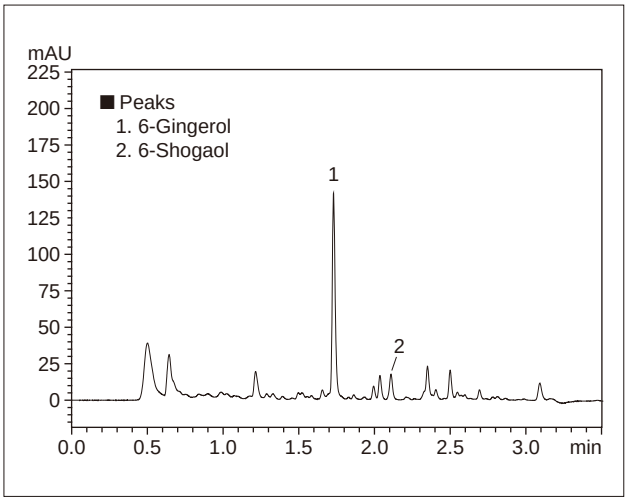


Fig. 1.33.4 Chromatogram of Ginger Extract

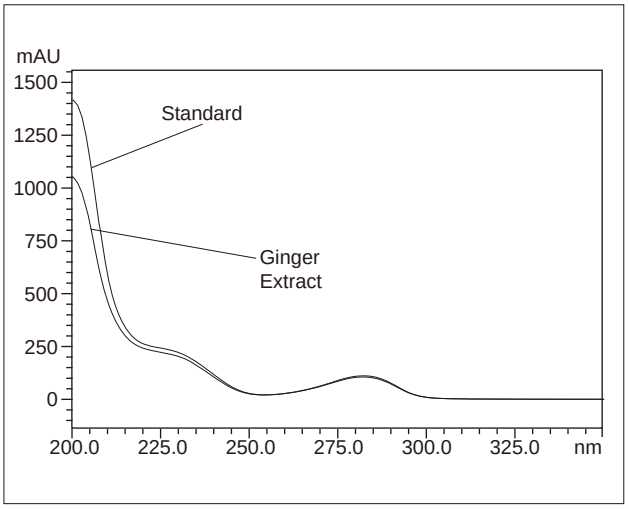


Fig. 1.33.5 Spectra of 6-Gingerol

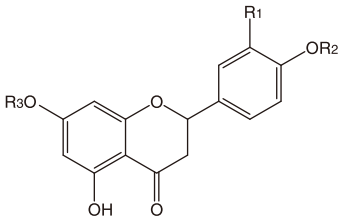
1.34 High Speed Analysis of Flavanones in Citrus Juice (1) - LC

■Explanation

Here we introduce an example of high-speed analysis of 6 flavanones. The LCMS-2010EV mass spectrometer was added to the Prominence UFLC/SPD-M20A configuration, and the analysis of minor components were verified using UV spectra as well as mass spectra.

■Analysis of 6 Flavanones

Fig. 1.34.1 shows the structures of the 6 flavanones analyzed here. Fig. 1.34.2 shows the results of simultaneous analysis of a standard mixture of the 6 flavanones using the SPD-M20A.



	R_1	R_2	R_3
Narirutin	H	H	Rutinose
Naringin	H	H	Neohesperidose
Hesperidin	OH	CH_3	Rutinose
Neohesperidin	OH	CH_3	Neohesperidose
Naringenin	H	H	H
Hesperetin	OH	CH_3	H

Fig. 1.34.1 Structures of the Flavanones

■Analytical Conditions

Column : Shim-pack XR-ODS (75 mm L. x 2.0 mm I.D. 2.2 µm)
Mobile Phase : A: 10 mmol/L Ammonium Formate Buffer (pH3.7) / Acetonitrile = 9/1 (v/v)
B: 10 mmol/L Ammonium Formate Buffer (pH 3.7) / Acetonitrile = 2/8 (v/v)
Gradient Elution Method
Time Program : B 0 % (0.00 min) → 50 % (5.00 min) → 100 % (5.01-6.00 min) → 0 % (6.01-9.00 min)
Flowrate : 0.4 mL/min with Semi-micro mixer
Column Temp. : 35 °C
Injection Volume : 2 µL
Detection : SPD-M20A at 285 nm
Flow Cell : Semi-micro cell

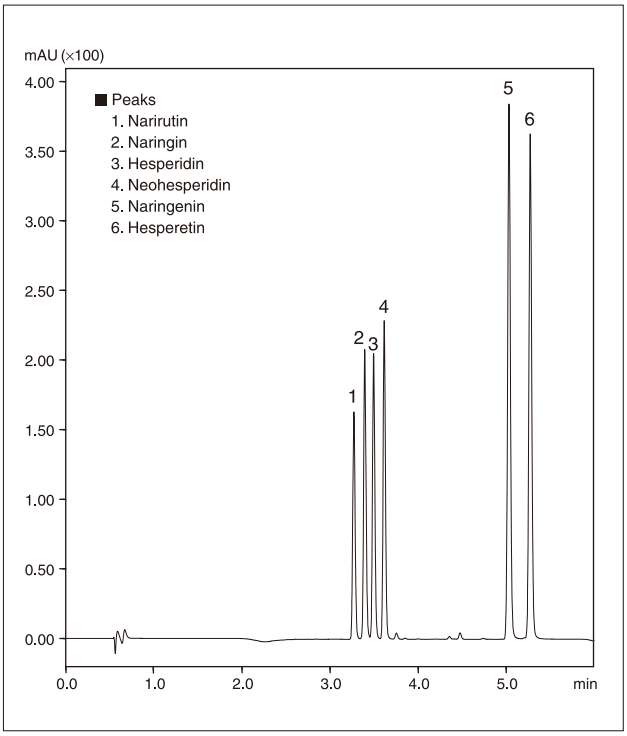


Fig. 1.34.2 Chromatogram of a Standard Mixture of 6 Flavanones (100 mg/L each, 2 µL injected)

1.34 High Speed Analysis of Flavanones in Citrus Juice (2) - LC

■Analysis of Grapefruit Juice

Fig. 1.34.4 and Fig. 1.34.5 show the results of analysis of commercial 100 % grapefruit juice using the SPD-M20A and LCMS-2010EV. Sample preparation was conducted using the procedure described in Fig. 1.34.3, and 2 µL was injected. From the chromatogram, it is evident that hesperidin and neohesperidin are included along with narirutin and naringin. However, the qualitative information is further supplemented by comparing the UV spectra and mass spectra. In addition, since the *m/z* 507 ion in the hesperidin mass spectrum is absent from standard solution mass spectrum, it is presumed to be an unknown compound. Even in this type of situation, monitoring of the *m/z* 609 ion can provide very accurate quantitation.

■LC/MS Analytical Conditions

Probe Voltage	: -3.5 kV (ESI-Negative Mode)
Nebulizing Gas Flow	: 1.5 L/min
Drying Gas Pressure	: 0.1 MPa
CDL, Q-array Voltages	: Using default values
CDL Temp.	: 250 °C
Block Heater Temp.	: 200 °C
Scan Range	: *m/z* 100 - 650

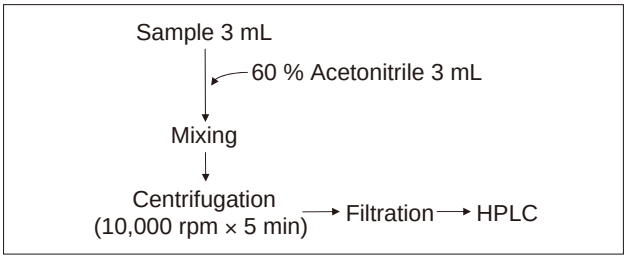


Fig. 1.34.3 Preparation of Citrus Juice Sample

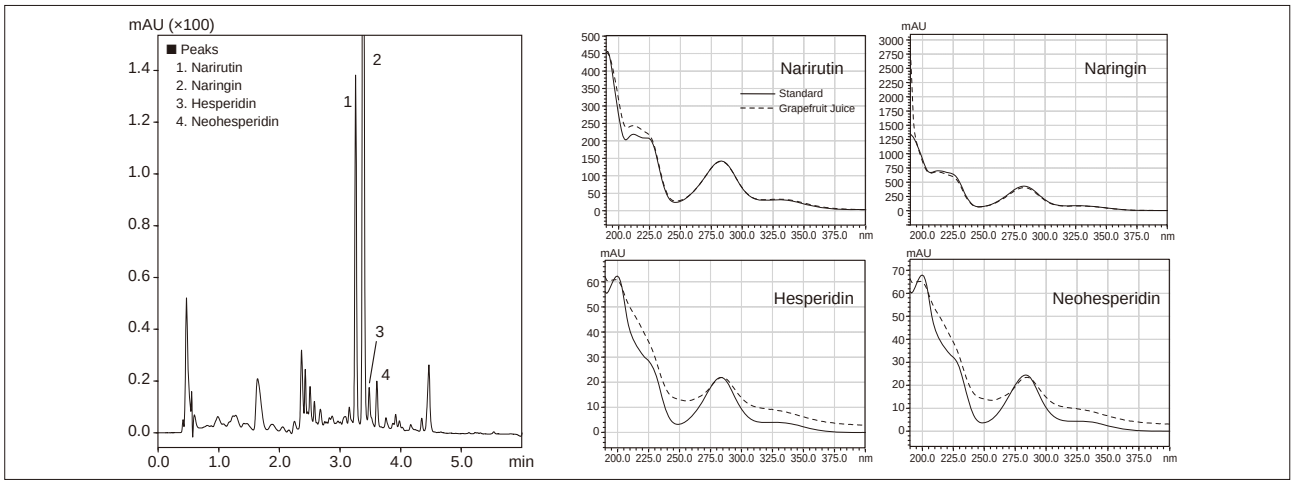


Fig. 1.34.4 UV Chromatogram and Spectra of Flavanones in Grapefruit Juice (2 µL injected)

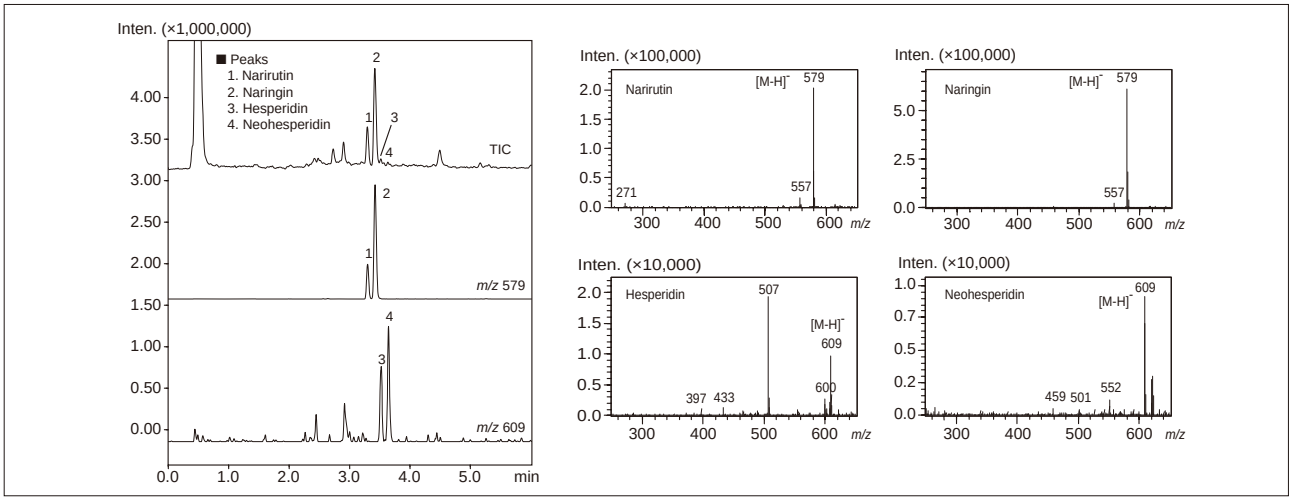


Fig. 1.34.5 Mass Chromatograms and Mass Spectra of Flavanones in Grapefruit Juice using Scan Mode (2 µL injected)

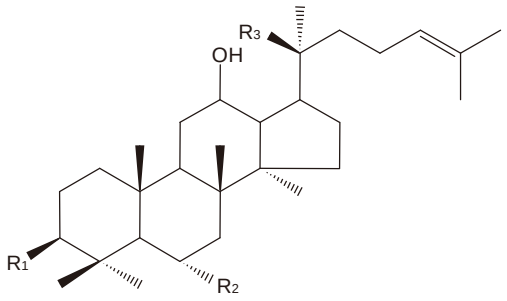
1.35 High Speed Analysis of Ginsenosides in Ginseng (1) - LC

■Explanation

Ginseng is a widely used herbal medicine with a number of reported health benefits including stress reduction, building resistance to disease, and promoting concentration and memory function. Compounds called ginsenosides are believed to be the active constituents behind ginseng's efficacy. Analysis of ginsenosides by HPLC has traditionally been a relatively time-consuming process due to the time required for separation of these structurally similar analytes as well as their separation from complex contaminants. Here we introduce an example of the analysis of ginsenosides in ginseng using the ultra fast LC system "Prominence UFLC" with the Phenomenex Synergi 2.5 µm Polar-RP high-speed, high-resolution column.

■Analysis of Standard Solution

The structural formulas of the 5 ginsenosides that are the subject of determination in this analysis are shown in Fig. 1.35.1. Here, separation of the ginsenosides Rg1 and Re in particular was conducted efficiently using the high-speed, high-resolution Phenomenex Synergi 2.5 µm Polar-RP (particle diameter 2.5 µm) column. Fig. 1.35.2 shows the results of analysis of a solution (60 % methanol aqueous solution) of the 5 ginsenosides, each present at 50 mg/L in the 2 µL sample.



Compound	R_1	R_2	R_3
Ginsenoside Rg1	OH	O-Glc	O-Glc
Ginsenoside Re	OH	O-Glc(2-1)Rha	O-Glc
Ginsenoside Rb1	O-Glc(2-1)Glc	H	O-Glc(6-1)Glc
Ginsenoside Rc	O-Glc(2-1)Glc	H	O-Glc(6-1)Araf
Ginsenoside Rd	O-Glc(2-1)Glc	H	O-Glc

Glc : β-D-glucose
Rha : α-L-rhamnose
Araf : α-L-arabinose (furanose)

Fig. 1.35.1 Structures of Ginsenosides

■Analytical Conditions

Column : Synergi 2.5 µm Polar-RP 100Å (50 mm L. × 2.0 mm I.D., 2.5 µm)
Mobile Phase : A: Water
B: Acetonitrile
Gradient Elution Method
Time Program : B 15 % (0 min) → 20 % (3 min) → 25 % (4 min) → 30 % (8 min)
Flowrate : 0.6 mL/min
Column Temp. : 35 °C
Injection Volume : 2 µL
Detection : SPD-20A at 203 nm
Flow Cell : Semi-micro cell

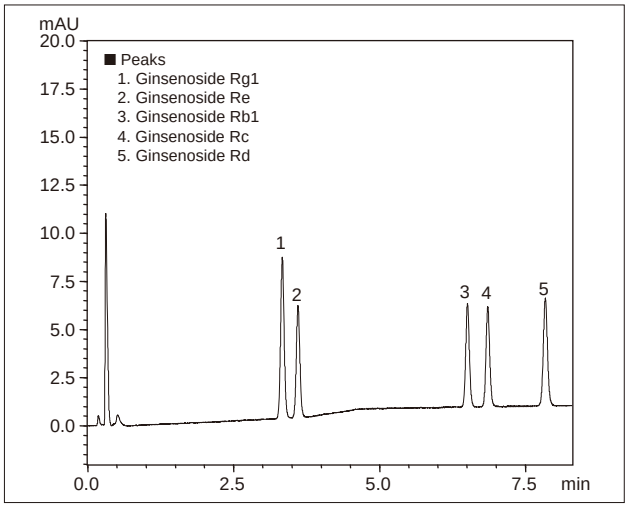


Fig. 1.35.2 Chromatogram of a Standard Mixture of 5 Ginsenosides (50 mg/L each)

1.35 High Speed Analysis of Ginsenosides in Ginseng (2) - LC

■Analysis of Powdered Ginseng

Fig. 1.35.3 shows the preparation procedure for herbal medicines as described in the Japanese Pharmacopeia. Fig. 1.35.4 shows the results of analysis of commercial ginseng powder, using a 2 µL injection of the sample prepared using the process shown in Fig. 1.35.3. Fig. 1.35.5 shows the procedure in which solid phase extraction (SPE) is incorporated in the sample preparation procedure of Fig. 1.35.3, using a reversed phase sorbent cartridge (Phenomenex "strata-X"), and the results of that analysis are shown in Fig. 1.35.6. Compared to the results of Fig. 1.35.4, it is clear that the high-polarity contaminants are effectively removed during the SPE step.

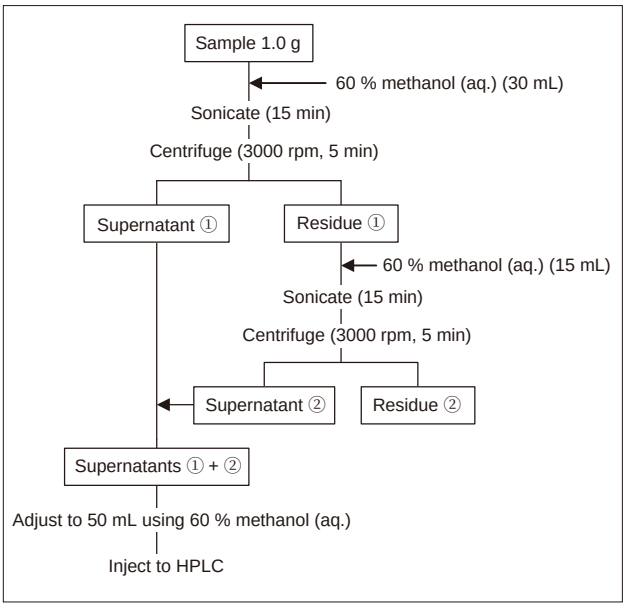


Fig. 1.35.3 Sample Preparation 1

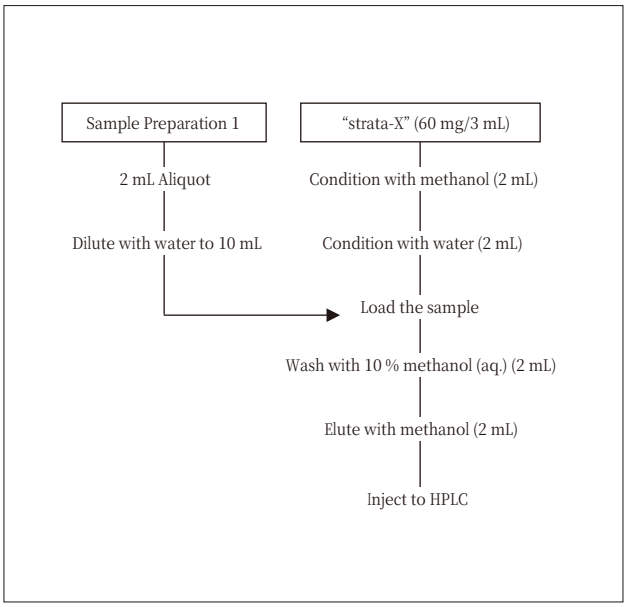


Fig. 1.35.5 Sample Preparation 2

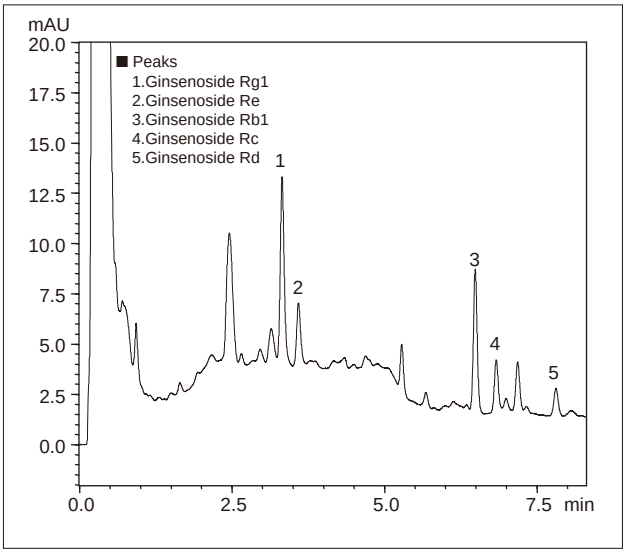


Fig. 1.35.4 Chromatogram of Powdered Ginseng (Using Sample Preparation 1)

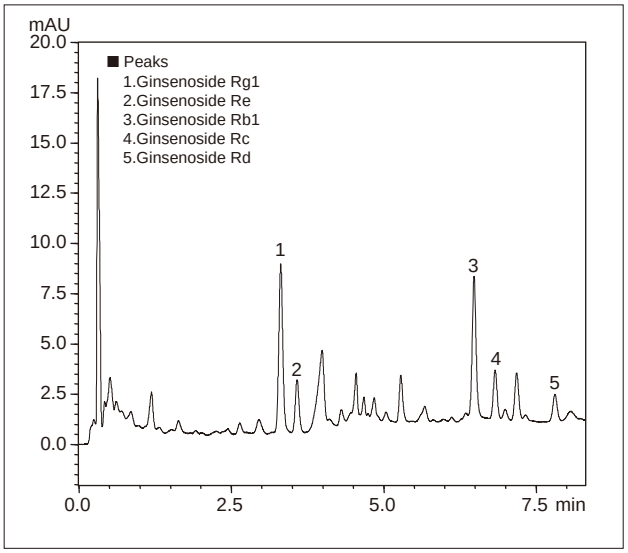


Fig. 1.35.6 Chromatogram of Powdered Ginseng (Using Sample Preparation 2)

[Reference]
The 15th Revision of the Japanese Pharmacopeia (Society of Japanese Pharmacopeia)

1.36 High Speed Analysis of Lutein and Zeaxanthin (1) - LC

■Explanation

Lutein and zeaxanthin are types of carotenoids, and are constituents of marigold pigment, a food additive which also occurs naturally in various foods. Recent studies have suggested that these substances may be effective in preventing cataracts and age-related macular degeneration syndrome (AMD).
Here we introduce an example of analysis of lutein and zeaxanthin in marigold supplement extract using the ultra fast LC "Prominence UFLC" with the SPD-M20A photodiode array detector.

■Analysis of Standard Solution

Fig. 1.36.1 shows the structures of lutein and zeaxanthin, which reveals that these 2 compounds are structurally similar, and in fact, structural isomers. Complete separation of such substances generally requires the use of reversed phase chromatography. In order to achieve more efficient separation through improved selectivity, we added tetrahydrofuran to the water / methanol mobile phase. In addition, use of the Shim-pack XR-ODS high speed, high resolution column (particle diameter 2.2 µm) allows the analysis time to be shortened to about 1/4 the time achieved with the conventational Shim-pack VP-ODS (particle diameter 4.6 µm), while maintaining the same resolution.

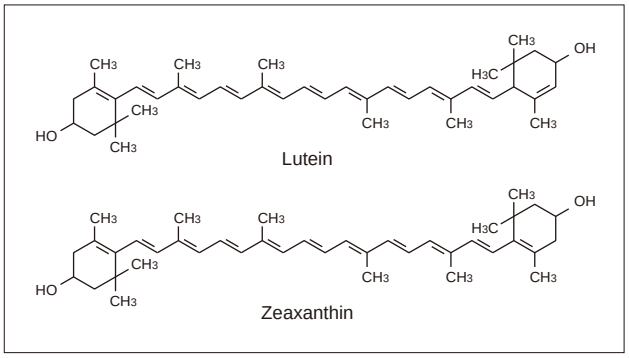


Fig. 1.36.1 Structures of Lutein and Zeaxanthin

■Analytical Conditions

Column : Shim-pack XR-ODS (75 mm L. x 3.0 mm I.D., 2.2 µm)
Shim-pack VP-ODS (150 mm L. x 4.6 mm I.D., 4.6 µm)
Mobile Phase : A : Methanol / Tetrahydrofuran / Water = 45/30/25 (v/v/v)
B : Tetrahydrofuran
Gradient Elution Method
Time Program : [XR-ODS]
B 0 % (0.00-4.50 min) → 100 % (4.51-5.50 min) → 0 % (5.51-7.5 min)
[VP-ODS]
B 0 % (0.00-15.50 min) → 100 % (15.51-20.00 min) → 0 % (20.01-25 min)
Flowrate : 0.8 mL/min (XR-ODS), 1.0 mL/min (VP-ODS)
Column Temp. : 50 °C
Injection Volume : 2 µL (XR-ODS), 5 µL (VP-ODS)
Detection : SPD-20A at 450 nm
Flow Cell : Semi-micro cell (XR-ODS)
Conventional cell (VP-ODS)

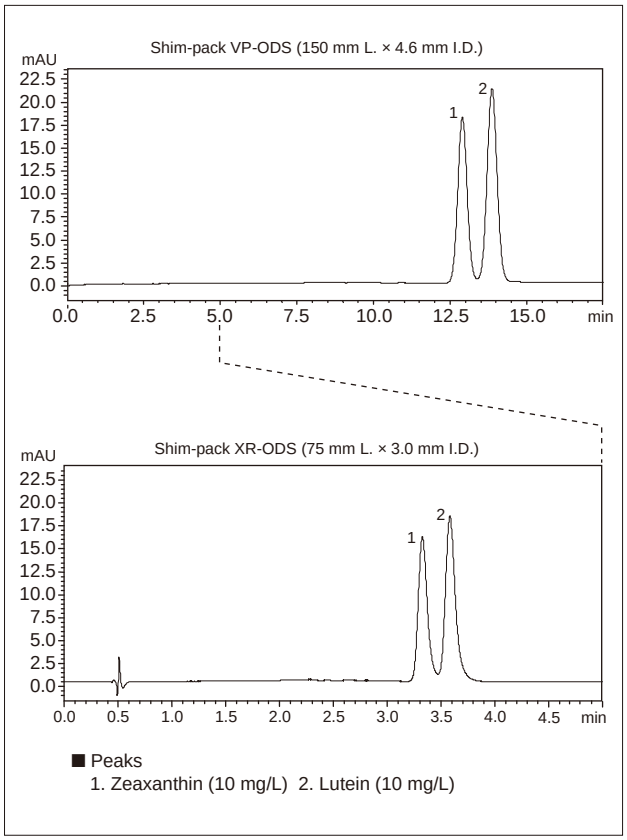


Fig. 1.36.2 Chromatograms of Lutein and Zeaxanthin Standard Solution

1.36 High Speed Analysis of Lutein and Zeaxanthin (2) - LC

■Analysis of Dietary Supplement

Fig. 1.36.3 shows the analysis results of commercial marigold extract contained in a dietary supplement (capsule form). Fig. 1.36.4 shows the sample preparation procedure. Fig. 1.36.5 shows overlay spectra of standard and sample solutions of lutein and zeaxanthin in a dietary supplement, respectively. In addition, Fig. 1.36.6 shows a 3-D plot of the dietary supplement.

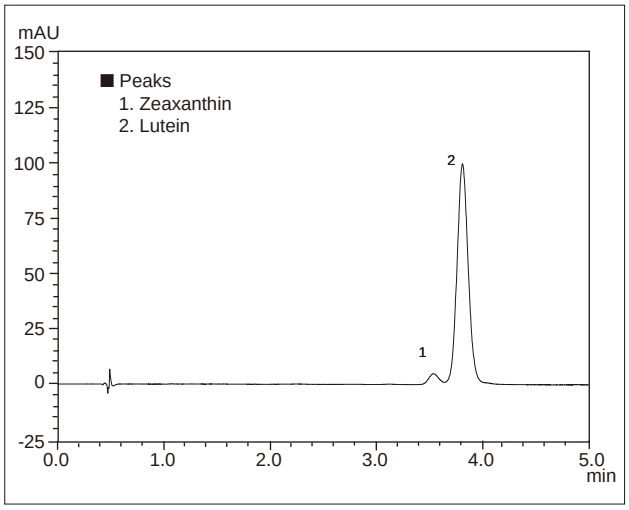


Fig. 1.36.3 Chromatogram of Dietary Supplement

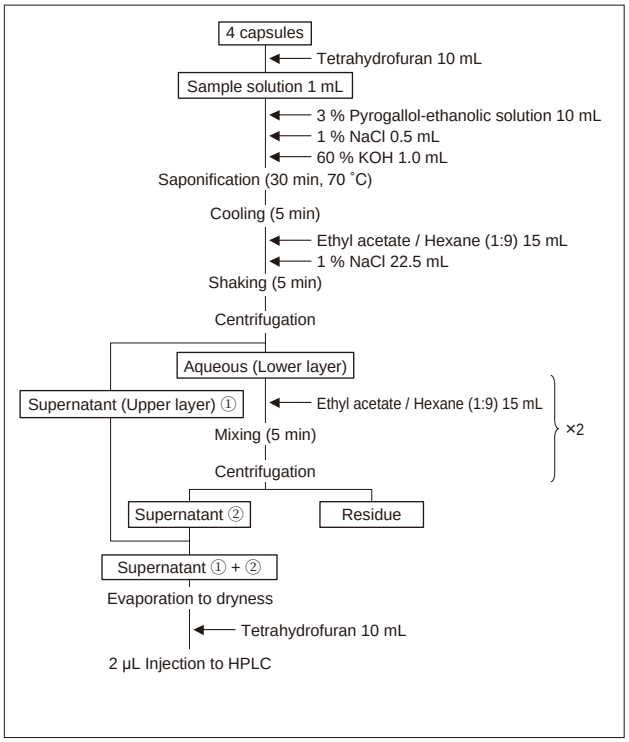


Fig. 1.36.4 Sample Preparation

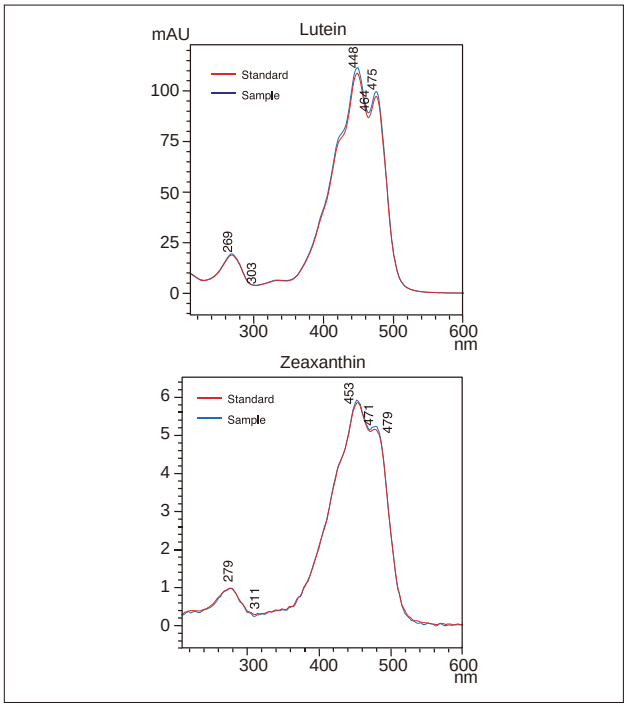


Fig. 1.36.5 Spectra of Lutein and Zeaxanthin

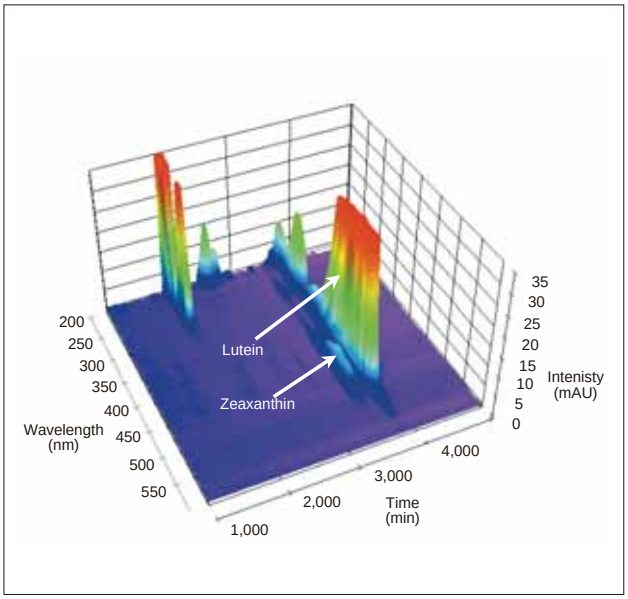


Fig. 1.36.6 3-D Plot of Dietary Supplement

1.37 Determination of Coenzyme Q10 in Food - LC

■Explanation

In Japan, coenzyme Q10 has traditionally been used as a pharmaceutical for improving myocardial metabolism. In accordance with revisions to the Food and Medicine Differentiation List (Pharmaceuticals and Food Safety Bureau, Ministry of Health, Labour and Welfare, Japan) in 2001, coenzyme Q10 was moved to the food section of the list. It is now the focus of attention as a food supplement. According to the Japanese Pharmacopoeia, which lists coenzyme Q10 under the pharmacological name, "Ubidecarenone", the recommended analysis method for coenzyme Q10 is the HPLC method. Here we introduce an analysis of coenzyme Q10 in commercially available food products using the Prominence Photodiode Array UV-Vis detector SPD-M20A.

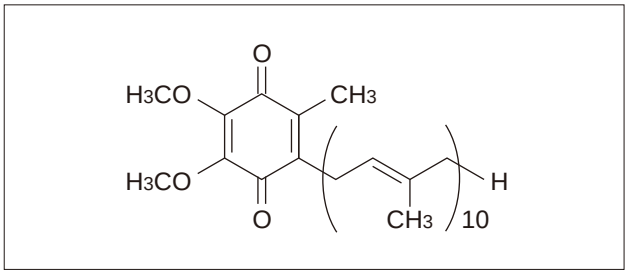


Fig. 1.37.1 Structure of Coenzyme Q10

■Analysis of Food Sample

Fig. 1.37.2 shows the resulting chromatogram, using a photodiode array detector, of a food sample (capsule) containing coenzyme Q10. The sample was dissolved* in ethanol (10 g/L) and the solution was filtered through a membrane filter (0.45 µm) before injection (5 µL). Fig. 1.37.3 shows comparison of the spectra of coenzyme Q10 in the standard solution and that of corresponding peak in the sample solution. We can see that the spectra closely match. Using a photodiode array detector makes it easy to obtain qualitative information from the UV absorption spectrum.

*A high concentration sample was used in this measurement. However, dilution by a factor of 100 is recommended for routine analytical in order to reduce load on the column.

■Analytical Conditions

Column	: Shim-pack FC-ODS (75 mm L. x 4.6 mm I.D.)
Mobile Phase	: Methanol / Ethanol = 13/7 (v/v)
Flowrate	: 1.5 mL/min
Column Temp.	: 40 °C
Injection Volume	: 5 µL
Detection	: SPD-20A at 275 nm
	Slit Width : 8 nm
	Cell Temp. : 40 °C

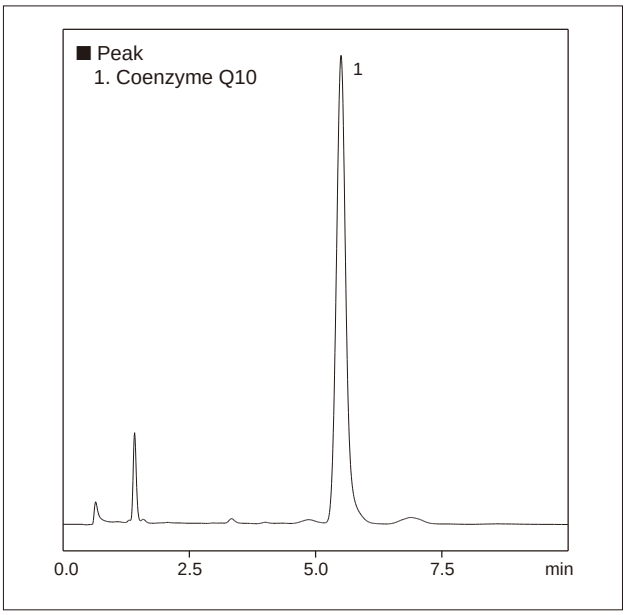


Fig. 1.37.2 Chromatogram of Food Sample (Capsule)

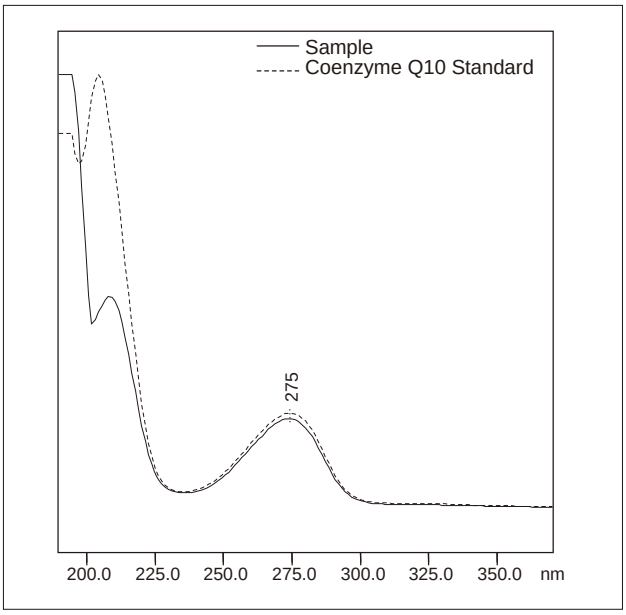


Fig. 1.37.3 UV Spectra of Coenzyme Q10

2. Food Additives

2.1 Analysis of Food Preservatives (1) - GC/MS

■Explanation

Food preservatives are used to prevent adverse changes and rotting of foods by suppressing the proliferation of microorganisms; they are used in accordance with the properties of the food to which they will be added. Many of the analytical methods used for analyzing food preservatives employ extraction of the compounds of interest by the steam distillation technique, followed by quantitative analysis using high performance liquid chromatography (HPLC). Gas chromatography can also be used for measurement of many compounds used as food preservatives, including benzoic acid, sorbic acid, methyl *p*-hydroxybenzoate (PHBA methyl), ethyl *p*-hydroxybenzoate (PHBA ethyl), propyl *p*-hydroxybenzoate (PHBA propyl), and butyl *p*-hydroxybenzoate (PHBA butyl), and therefore, gas chromatography / mass spectrometery (GC/MS) is an effective analysis technique for verifying the detection results obtained by HPLC. Here we introduce an example of GC/MS analysis of six types of food preservatives.

■Analytical Conditions

Instrument : GCMS-QP2010 Plus
-GC-
Column : Rtx-5MS (30 m × 0.25 mm I.D. df = 0.25 µm)
Injection Temp. : 60 °C - 15 °C/min - 280 °C (20 min)
Carrier Gas : He, 45.0 cm/sec
Carrier Gas Mode : Constant Linear Velocity Mode
Injection Temp. : 250 °C
Injection Method : Split Injection
Split Ratio : 1:10
Injection Volume : 1 µL

-MS-
I.F. Temp. : 260 °C
I.S. Temp. : 230 °C
Ionization Method : EI
Scan Range : *m/z* 40-300
Scan Interval : 0.3 sec

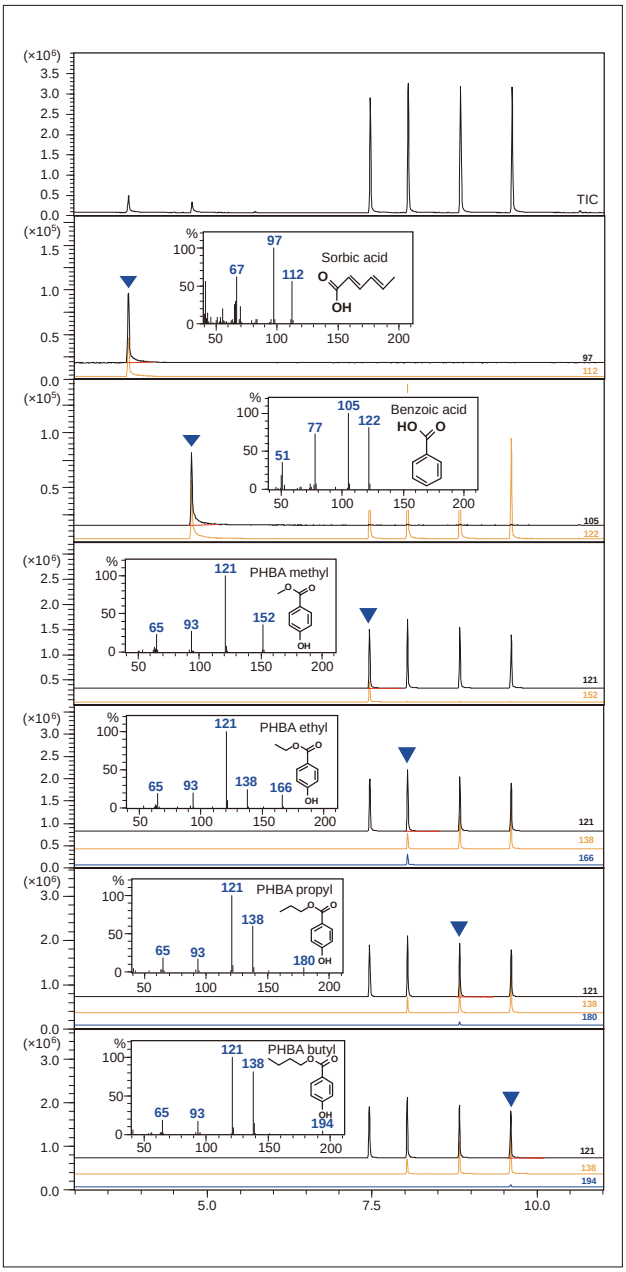


Fig. 2.1.1 Chromatograms of 6 Preservatives

2.1 Analysis of Food Preservatives (2) - GC/MS

■Results

Standard Samples

Each of six preservatives was used to prepare a 10 mg/L standard solution. The resulting chromatograms are shown in Fig. 2.1.1. The uppermost data is the TIC chromatogram, and the remaining chromatograms are the mass chromatograms corresponding to the characteristic *m/z* of each of the substances; the mass spectrum of each substance is also shown on the corresponding mass chromatogram. Although sensitivities for benzoic acid and sorbic acid were lower than those for the PHBA esters, the peaks for benzoic acid and sorbic acid were clearly detected.

Soft Drinks

Many commercially available soft drinks contain sodium benzoate as a preservative, and one such soft drink in which sodium benzoate was indicated as a preservative ingredient was analyzed as part of this study. The pretreatment consisted only of diluting the sample 50 to 1 with ethanol; the diluted sample was analyzed directly. The analytical results indicate the detection of benzoic acid in the mass chromatogram (Fig. 2.1.2).

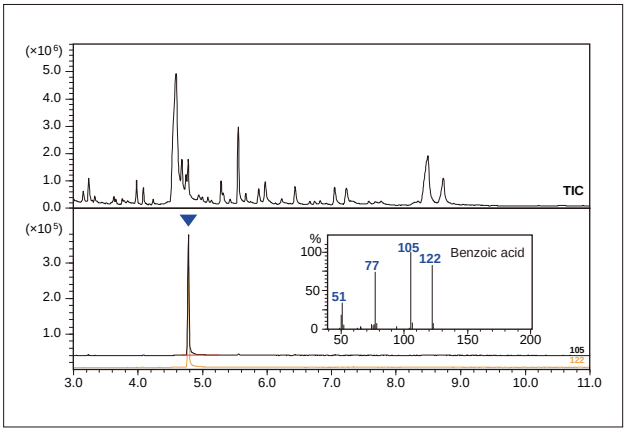


Fig. 2.1.2 Result of Soft Drink Analysis

Liquid Condiment

In Japan, the use of PHBA esters is permitted in liquid condiments such as soy sauce. A liquid condiment sample spiked with 5 µg/g of PHBA esters was also analyzed as part of this study. For the pretreatment, the sample was diluted 10 to 1 with ethanol and centrifuged for 5 minutes at 1000 rpm. The precipitate was removed, and the supernatant solution was analyzed without any further pretreatment. The analytical results are shown in Fig. 2.1.3. The mass chromatograms of the added PHBA esters demonstrate that they were clearly detected.

Many chromatographic peaks were observed in the TIC chromatograms of both the soft drink and the liquid condiment. Although the compounds of interest are difficult to observe in the TIC, the target substances are clearly detected in the corresponding mass chromatograms. Furthermore, mass spectra of the preservatives were easily obtained using spectrum subtraction. These results demonstrate that the preservative could be detected not only in the standard sample but in the actual samples as well using a simple pretreatment procedure. Clearly the results of this study show that analysis by GC/MS is effective as a confirmation technique for these substances.

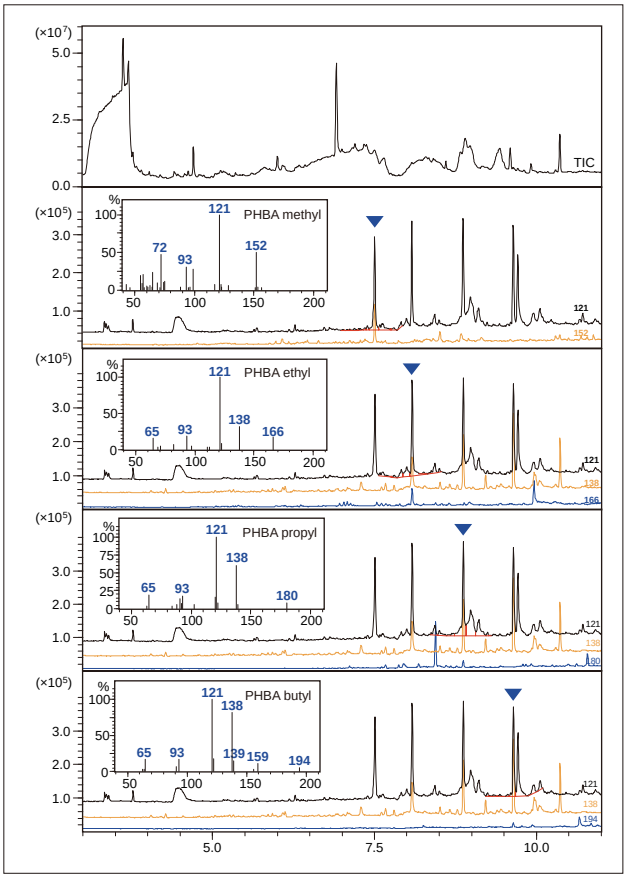


Fig. 2.1.3 Chromatograms of Soy Sauce Spiked with 4 kind of Preservatives

2.2 High Speed Analysis of Benzoic Acid and Sorbic Acid - LC

■Explanation

Benzoic acid (as sodium benzoate), sorbic acid (as potassium sorbate), and sodium dehydroacetate, etc. are used as preservatives in food with the aim of preventing food poisoning and the proliferation of microorganisms. Determination of these compound levels in food is generally conducted using HPLC. Here we introduce an example of high speed analysis of benzoic acid, sorbic acid, and dehydroacetic acid using the Prominence UFLC ultra fast LC system with the Shim-pack XR-ODS high-speed, high-resolution column.

■Analytical Conditions

Column	: Shim-pack XR-ODS (75 mm L. × 3.0 mm I.D., 2.2 μm)
Mobile Phase	: 5 mmol/L (Sodium) Citrate Buffer (pH4.2) / Acetonitrile = 3/1 (v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 4 μL
Detection	: SPD-20A at 230 nm
UV Cell	: Semi-micro cell

■Analysis of a Soft Drink

Fig. 2.2.1 shows the results of analysis of a commercial soft drink. A 10-fold dilution of the soft drink was prepared using distilled water, and after filtering it through a membrane filter (0.2 μm pore diameter), 4 μL were injected.

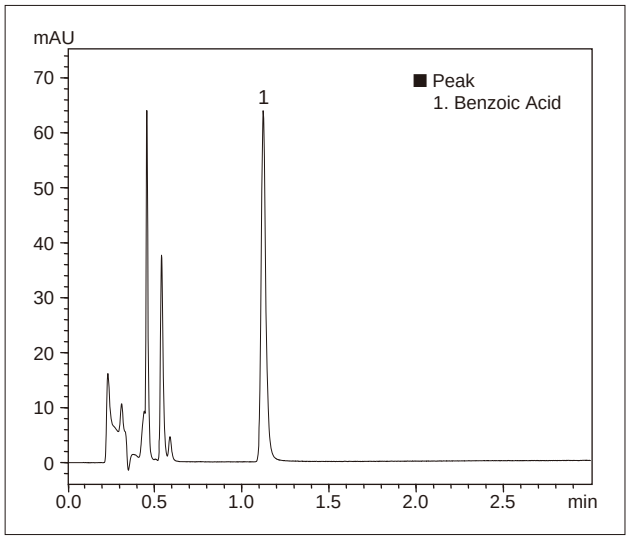


Fig. 2.2.1 Chromatogram of a Soft Drink

■Analysis of Pickle Juice

Fig. 2.2.2 shows the results of analysis of pickle juice. After centrifuging the pickle juice, the supernatant was collected and diluted 10 times using distilled water, filtered through a membrane filter (0.2 μm pore diameter), and 4 μL were injected.

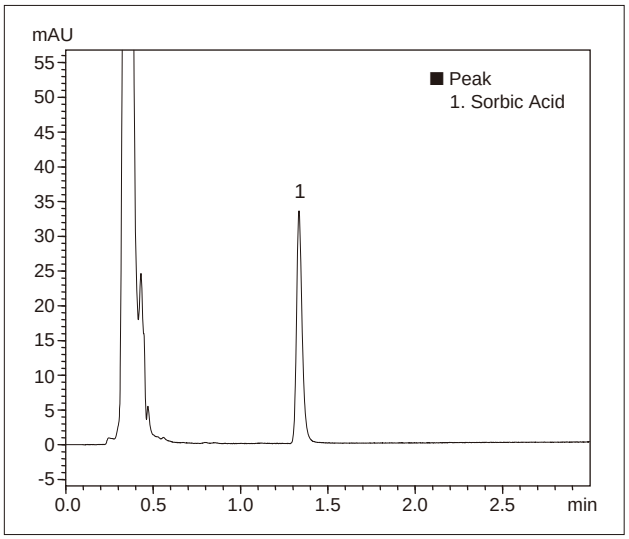


Fig. 2.2.2 Chromatogram of Pickle Juice

2.3 Analysis of Natamycin in Cheese - LC

■Explanation

Natamycin is a polyene macrolide antibiotic that specifically inhibits the growth of mold and yeast, and was specified as a food additive (surface treatment agent for cheese) to the "Japanese Food Additive Analysis Methods" revised on November 28, 2005[1], and the HPLC natamycin analytical method was added[2]. Here we introduce an example of analysis of natamycin in cheese using HPLC.

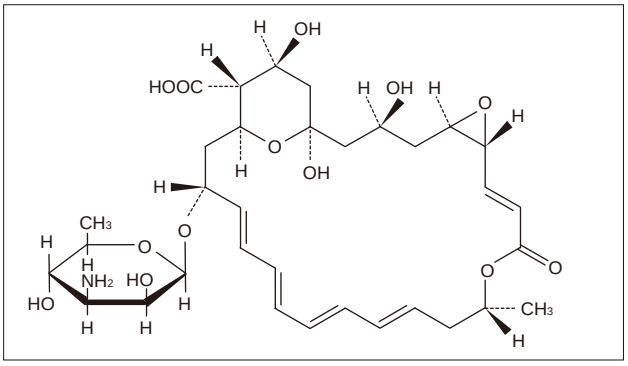


Fig. 2.3.1 Structure of Natamycin

■Analysis of Natamycin in Cheese

Two types of commercial cheese samples were prepared according to the procedure shown in Fig. 2.3.2, and analysis was conducted. Each of these samples were spiked with an appropriate amount of natamycin standard to achieve a concentration of 0.5 mg/kg. Fig. 2.3.3 and Fig. 2.3.4 show the respective analytical results.

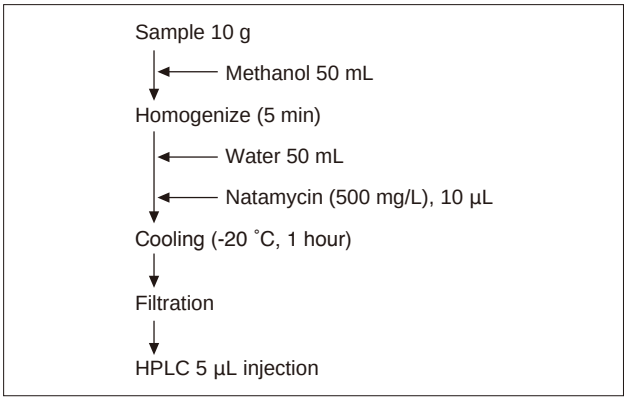


Fig. 2.3.2 Sample Preparation

■Analytical Conditions

Column : Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D.)
Mobile Phase : Methanol/Water/Acetic Acid = 50/50/5 (v/v/v)
Flowrate : 1.0 mL/min
Column Temp. : 40 °C
Detection : SPD-20A at 304 nm

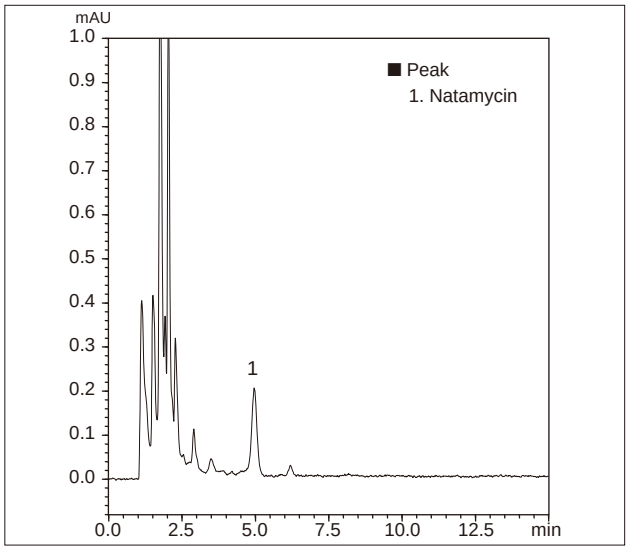


Fig. 2.3.3 Chromatogram of Cheese A (spiked with natamycin)

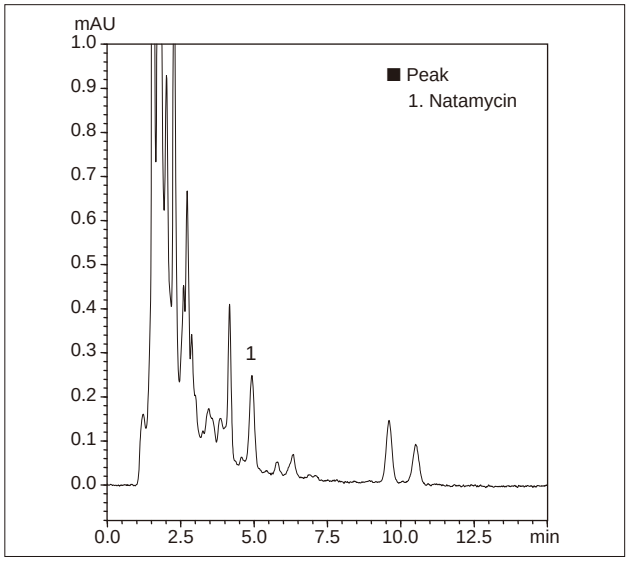


Fig. 2.3.4 Chromatogram of Cheese B (spiked with natamycin)

[References]

1) "Ministerial Ordinance Revising Part of the Enforcement Regulations of the Food Sanitation Law, and Partial Revision of Standards for Foods and Food Additives" (July 28, 2005 Ministry of Health, Labour and Welfare, Food Safety Supplement No. 1128002, Japan)
2) "Food Additive Analysis Methods" (July 28, 2005 Ministry of Health, Labour and Welfare, Food Safety Supplement No. 1128001, Japan)

2.4 Analysis of Food Antioxidants (1) - GC/MS

■Explanation

Food antioxidants serve to prevent the oxidation of food ingredients by becoming oxidized themselves instead. BHT (dibutylhydroxytoluene) and BHA (butylhydroxyanisol), which had become widely used as food antioxidants, are almost never used now because of concern related to their reported carcinogenicity. Instead, vitamin C and vitamin E have recently become widely used. The use of some food additives is not permitted in Japan, but many such substances are permitted in some countries. For example, the antioxidant TBHQ (*t*-butylhydroquinone) is not permitted to be used in Japan; however, since it may be used in many other countries, there is a possibility of it being included in food imports to Japan. Here we introduce the results of analysis of the antioxidants BHA, BHT and TBHQ using a gas chromatograph mass spectrometry (GC/MS). The antioxidants were added to a butter sample, and then extracted using a simple pretreatment process. In this analysis, the backflush technique was employed to prevent high-boiling point substances in the actual sample from being introduced into the detector.

■Results and Discussion

Analysis of Standard Sample

A sample solution consisting of 10 mg/L each of BHA, BHT and TBHQ in acetone was analyzed. The results are shown in Fig. 2.4.1. The TIC chromatogram is shown at the top, and the mass spectra of the respective constituent peaks and mass chromatograms at the characteristic *m/z* values are shown below. Each constituent peak was clearly detected.

Recovery Analysis of Spiked Components in Actual Sample

Recovery analysis was conducted for substances added to an actual sample of commercially available butter. After dissolving 100 mg of butter in 1 mL acetone, centrifugal separation (1000 rpm, 5 minutes) was conducted to precipitate the extract, and supernatant was collected and analyzed. For recovery testing, BHA, BHT and TBHQ were added to the butter sample to attain a final concentration of 5 mg/kg each (concentrations of BHA, BHT and TBHQ in sample following pretreatment were 0.5 mg/L, respectively). The resulting chromatogram is shown in Fig. 2.4.2. As in Fig. 2.4.1, the TIC chromatogram is shown at the top, and the mass spectra of the respective constituent peaks and mass chromatograms at the characteristic *m/z* values are shown below. In the actual sample as well, peak detection could be conducted selectively for the target constituents of the mass chromatograms at the characteristic *m/z* values. Mass spectra similar to those obtained using the standard solution were also obtained. The analysis was conducted 3 times to determine the repeatability of the quantitation values. The repeatability results and recoveries based on the average quantitation value with n=3 are shown in Table 2.4.1. Excellent repeatability and recovery were obtained for the actual sample.

■Analytical Conditions

Instrument	: GCMS-QP2010 Plus
-GC-	
Column	: Rtx-5MS (30 m × 0.25 mm I.D. df = 0.25 µm)
Column Temp.	: 60 °C - 15 °C/min - 280 °C (20 min)
Carrier Gas	: He (45 cm/sec, 174.3 kPa)
Carrier Gas Mode	: Constant Pressure Mode
Injection Temp.	: 250 °C
Injection Method	: Split Injection
Split Ratio	: 1:5
Injection Volume	: 1 µL
-MS-	
I.F. Temp.	: 260 °C
I.S. Temp.	: 230 °C
Ionization Method	: EI
Scan Range	: *m/z* 40-300
Scan Interval	: 0.3 sec

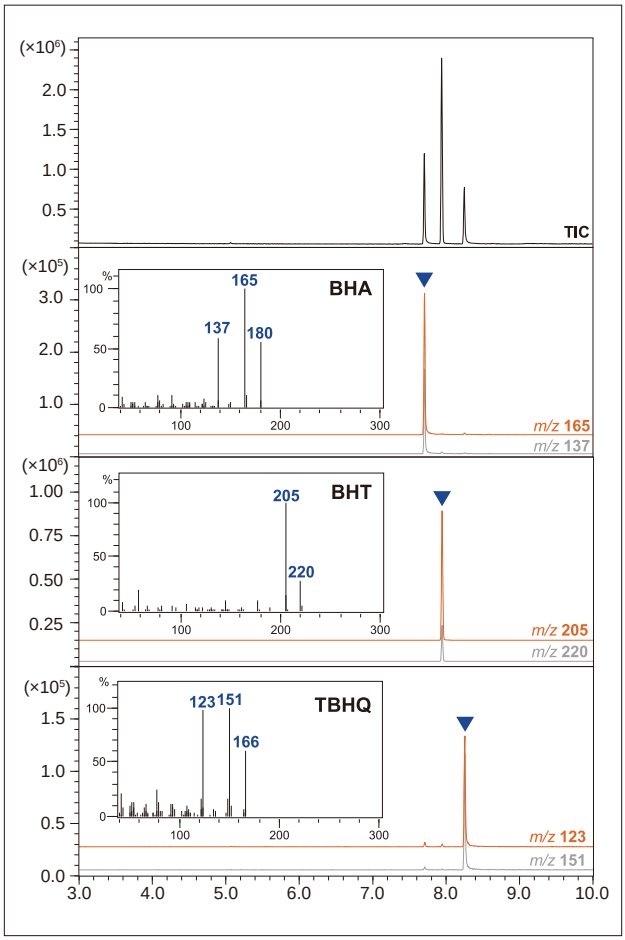


Fig. 2.4.1 Chromatograms of BHA, BHT and TBHQ (10 mg/L)

2.4 Analysis of Food Antioxidants (2) - GC/MS

Table 2.4.1 Results of Butter Sample Analysis

	Quantitation Values (mg/kg)				RSD (%)	Average Recovery Rate (%)
	1	2	3	Average		
BHT	6.4	6.2	5.8	6.1	4.7	123
BHA	4.7	4.6	4.6	4.6	1.3	93
TBHQ	5.9	5.9	5.3	5.7	6.4	114

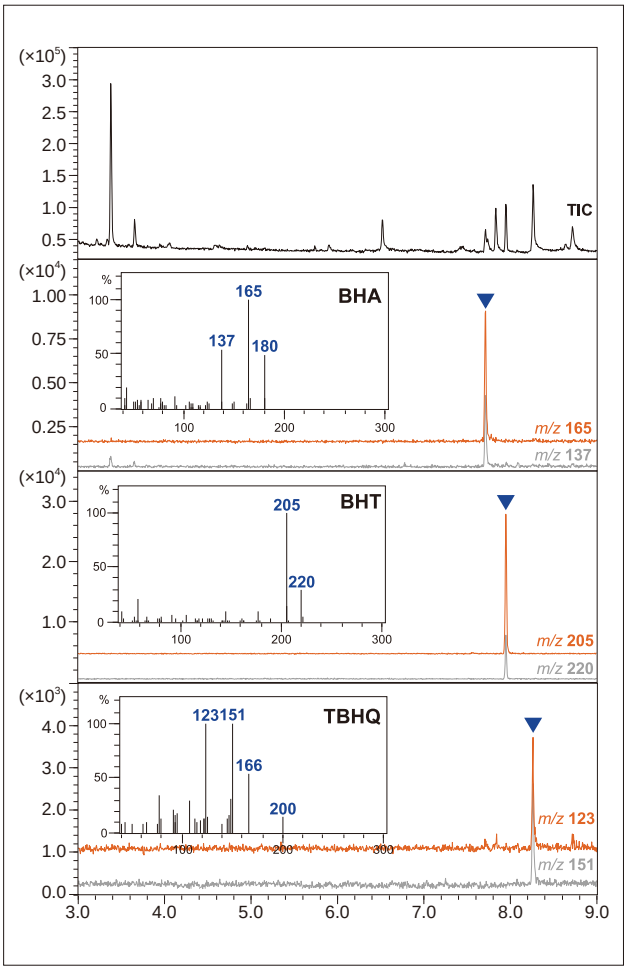


Fig. 2.4.2 Chromatograms of Butter Sample (5 mg/L)

Capillary Backflush System

In this investigation, actual sample analysis was conducted using a simple sample preparation process (minimal sample cleanup was performed after extraction). Therefore, most of the high-boiling point substances would typically be introduced into the GC/MS.Backflushing reverses the flow of carrier gas in the column to discharge high-boiling point substances from the split vent of the injection port. One of the benefits of backflushing is the reduced frequency of maintenance, since the high-boiling point substances are not introduced into the detector. In addition, high-boiling point substances may not be completely discharged from the column during the analysis. Residual high-boiling point substances in the column can cause such problems as reduced sensitivity and poor repeatability, so discharging them via the injection port provides an efficient means of removing them from the column while extending the life of the column. The backflush settings were implemented via time program, so that following elution of the target components (in this analysis, at 9 minutes), the split vent pressure was raised from 20 kPa to 200 kPa, and at the same time the column pressure was reduced to 20 kPa. This program can be set automatically using the backflush software utility. Fig. 2.4.3 shows a comparison of TIC chromatograms of butter analysis with and without the use of the backflush technique. The zoomed chromatograms in Fig. 2.4.3 display only the data acquired up to the completion of elution of BHA, BHT and TBHQ. Similar chromatograms were obtained when backflush was used and when it was not used. When backflushing was conducted, however, no peaks were detected after the 9 minutes corresponding to the time setting. Implementing the backflush prevented introduction of the high-boiling point substances into the detector, and provided a means of discharging them efficiently from the column.

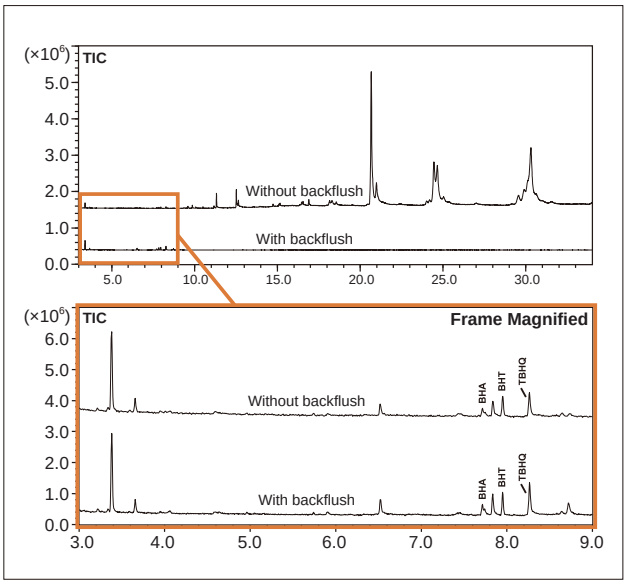


Fig. 2.4.3 Comparison of Butter Sample Analysis with/without Backflush

2.5 High Speed Analysis of Ascorbic Acid and Erythorbic Acid - LC

■Explanation

As an analytical method for determination of the food antioxidants ascorbic acid and erythorbic acid, the hydrophilic interaction liquid chromatography (HILIC) mode, a type of normal-phase chromatography using an amino column, is used in combination with an acetonitrile / aqueous mobile phase. Here we introduce an example of high-speed, high-resolution analysis of ascorbic acid and erythorbic acid using the Prominence UFLC ultra fast LC system with the high-speed analysis HILIC mode Phenomenex LUNA NH_2 column (particle diameter 3 µm).

■Analysis of Standard Solution

Fig. 2.5.1 shows the structural formulas of ascorbic acid and erythorbic acid. Fig. 2.5.2 shows the analysis of a standard mixture of ascorbic acid and erythorbic acid (20 mg/L each). The standard solution was prepared using an acetonitrile / water (8/2, v/v) solution, and 2 µL of this solution were immediately injected. When using the Phenomenex LUNA NH_2 column (particle diameter 3 µm), the analysis can be completed within 1.5 minutes using a 50 mm column while maintaining excellent resolution.

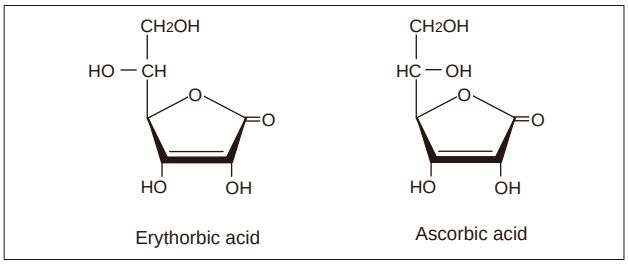


Fig. 2.5.1 Structural Formulas

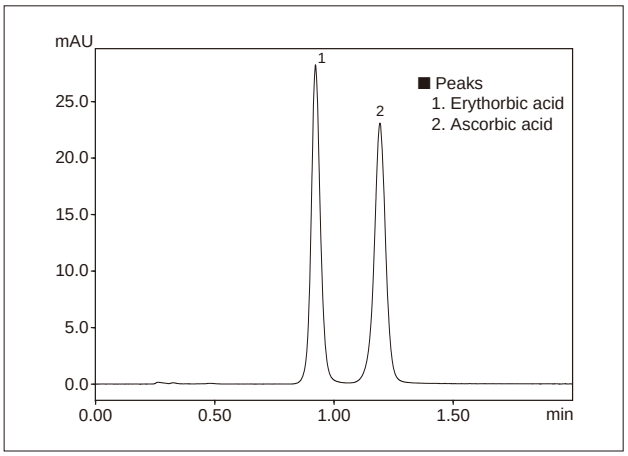


Fig. 2.5.2 Chromatogram of a Standard Mixture of Ascorbic Acid and Erythorbic Acid (20 mg/L each, 2 µL injected)

■Analytical Conditions

Column	: Phenomenex LUNA NH_2 (50 mm L. × 3.0 mm I.D., 3 µm)
Mobile Phase	: 50 mmol/L (Triethanolamine) Phosphate Buffer (pH 2.2) / Acetonitrile = 15/85 (v/v)
Flowrate	: 0.8 mL/min
Column Temp.	: 40 °C
Injection vol.	: 2 µL
Detection	: SPD-20AV at 240 nm

■Analysis of Soft Drinks

Figs. 2.5.3 and Fig. 2.5.4 show the analysis of commercial soft drinks. Soft drink A (vitamin-added soft drink) was diluted 10 times with distilled water, and further diluted 10 times with an acetonitrile / water (8/2, v/v) solution. Soft drink B (green tea beverage) was diluted 5 times with distilled water, and further diluted 5 times with an acetonitrile solution. Each of these solutions was then filtered through a membrane filter (pore diameter 0.2 µm), and 2 µL of each were injected for analysis.

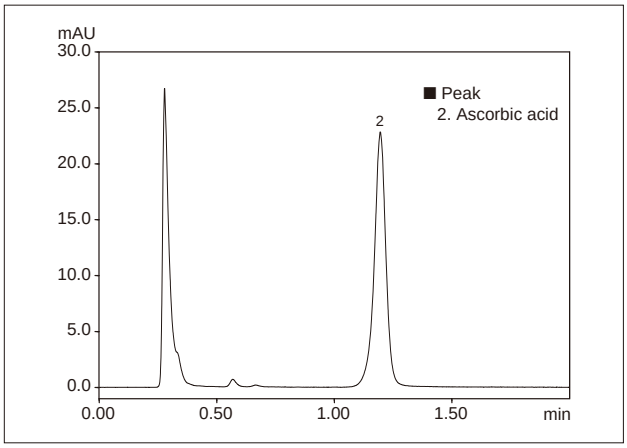


Fig. 2.5.3 Chromatogram of Soft Drink A (2 µL injected)

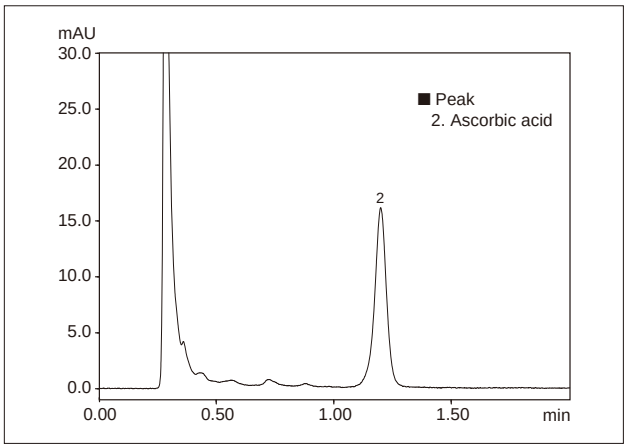


Fig. 2.5.4 Chromatogram of Soft Drink B (2 µL injected)

2.6 High Speed Analysis of Tocopherols - LC

■Explanation

Tocopherols (Vitamin E) consist of a class of nutrients that are not only used as food additives such as antioxidants and nutritional supplements, but are also included in natural ingredients. Tocopherols are known to exist in various forms, including the β-, γ-, and δ- isomers, not to mention α-tocopherol (Fig. 2.6.1). HPLC analysis of tocopherol isomers is typically conducted using normal phase chromatography combined with fluorescence detection. Here we conducted ultra fast analysis using the Shim-pack XR-SIL high-speed, high-resolution analytical column (particle diameter 2.2 µm) together with the RF-20Axs detector. Fig. 2.6.2 shows an example of analysis of a standard solution of 4 tocopherols (2 mg/L each) using the Shim-pack CLC-SIL (M) conventional column (particle diameter 5 µm) and the Shim-pack XR-SIL. The Shim-pack XR-SIL shortened the analysis time to less than one-fourth.

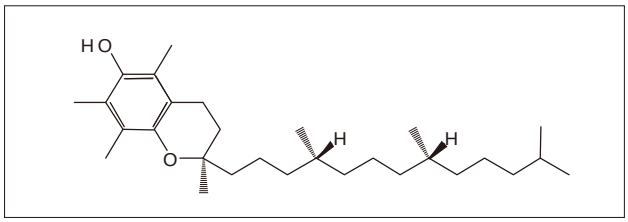


Fig. 2.6.1 Structure of α-Tocopherol

■Analytical Conditions

Column	: Shim-pack XR-SIL (75 mm L. × 3.0 mm I.D., 2.2 µm) Shim-pack CLC-SIL(M) (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase	: Hexane / 2-Propanol = 100/0.5 (v/v)
Flowrate	: 1.2 mL/min (XR-SIL), 1.0 mL/min (CLC-SIL(M))
Column Temp.	: 30 °C
Injection Volume	: 4 µL (XR-SIL), 10 µL (CLC-SIL(M))
Detection	: RF-20Axs Ex. at 298 nm, Em. at 325 nm
Cell Temp.	: 25 °C
Flow Cell	: Semi-micro cell (XR-SIL) Conventional cell (CLS-SIL(M))

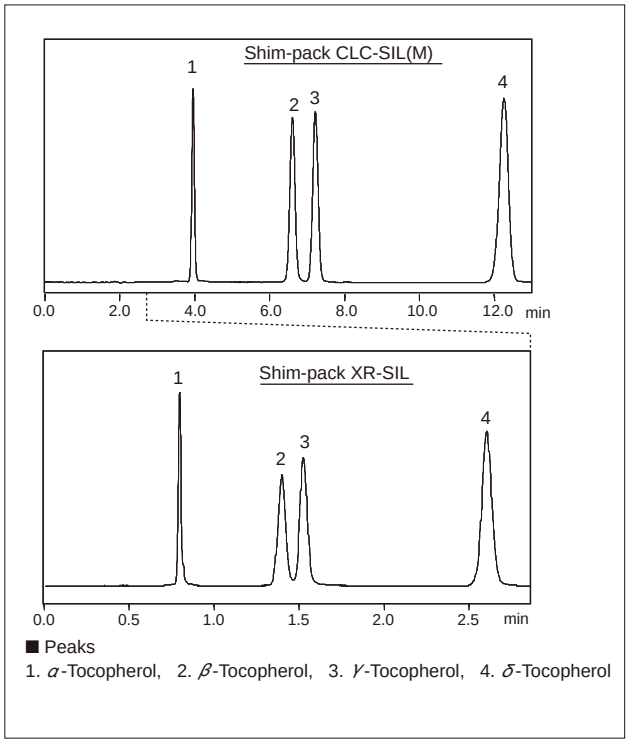


Fig. 2.6.2 Chromatograms of Standard Mixture of Tocopherols (2 mg/L each)

2.7 Simultaneous Determination of Sweeteners in Food (1) - LC

■Explanation

Several different low calorie sweeteners have been used in a variety of foods and beverages in recent years. These sweeteners present a distinct taste that set them apart from sucrose, glucose, fructose, etc., and the overall taste can be manipulated by mixing multiple sweeteners. Here we introduce an example of simultaneous analysis of 6 sweeteners using the ELSD-LT*II* Evaporative Light Scattering Detector.

■Analysis of Standard Solution

Fig. 2.7.1 shows the structural formulas of the 6 sweeteners analyzed for this experiment. Because the polarity varies greatly among these, analysis must be conducted by gradient elution. Moreover, because sucralose displays little UV absorption, simultaneous analysis was attempted using an evaporative light scattering detector (ELSD). Fig. 2.7.2 shows the chromatogram of the standard mixture. The 6 compounds were effectively separated without the use of an ion-pair reagent by using the Phenomenex Luna PFP (2) (particle size: 5 µm) column, which incorporates a pentafluorophenyl solid phase as the analytical column. It was confirmed that glucose and sucrose were eluted as unretained peaks.

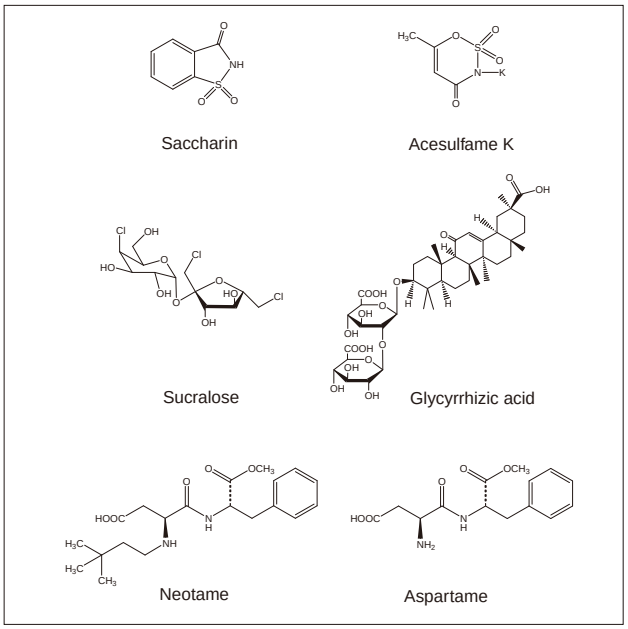


Fig. 2.7.1 Structures of 6 Sweeteners

■Analytical Conditions

Column : Phenomenex Luna PFP (2)
(150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase : A: 10 mmol/L Ammonium Formate Buffer (pH 3.7)
B: Acetonitrile
Gradient Elution Method
Time Program : B 5 % → 50 % (0.00-15.00 min)
→ 50 % (15.01-18.00 min)
→ 5 % (18.01-30.00 min)
Flowrate : 1.0 mL/min
Column Temp. : 40 °C
Injection Volume : 10 µL
Detection : ELSD-LT *II*
Temperature : 40 °C
Gain : 6
Nebulizer : Gas N_2
Gas Pressure : 350 kPa

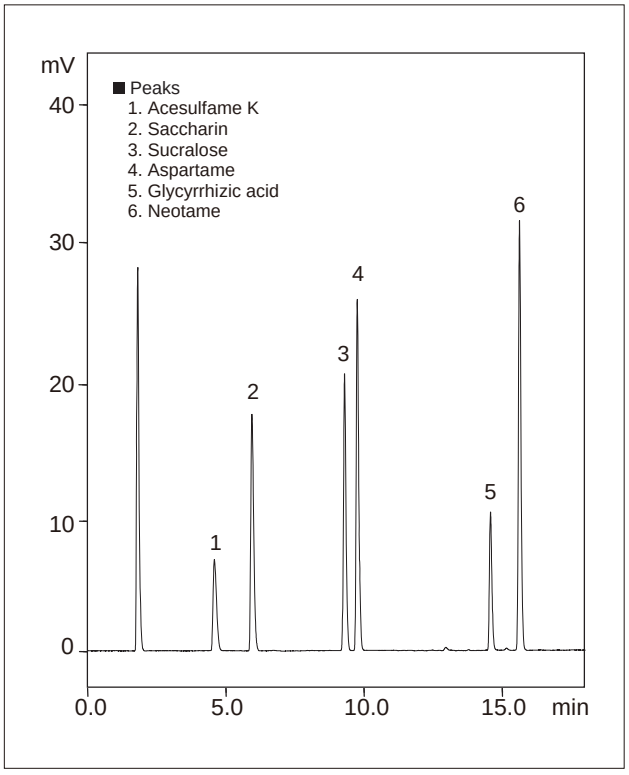


Fig. 2.7.2 Chromatogram of a Standard Mixture of 6 Sweeteners (100 mg/L each, 10µL injected)

2.7 Simultaneous Determination of Sweeteners in Food (2) - LC

■Analysis of Soft Drink

Fig. 2.7.3 to Fig. 2.7.5 show examples of analysis of commercial soft drinks. Using 100 mmol/L ammonium formate buffer solution, the low-sugar coffee and sports drink were diluted 5-fold and 2-fold, respectively, and after filtering through a 0.2 µm membrane filter for aqueous solutions, each sample was injected at a volume of 10 µL for analysis. For the carbonated beverage, after the carbon dioxide was removed by ultrasonic treatment, the same buffer solution was used to prepare a 5-fold dilution. Then, after filtering through a 0.2 µm membrane filter for aqueous solutions, 10 µL was injected.

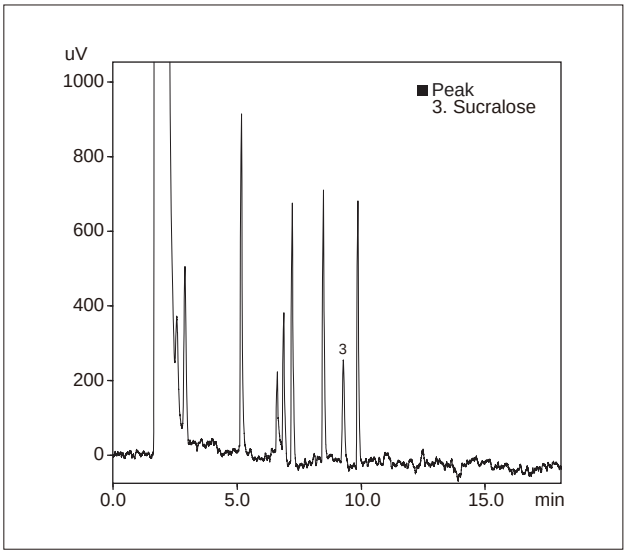


Fig. 2.7.4 Chromatogram of Sports Drink (10 µL injected)

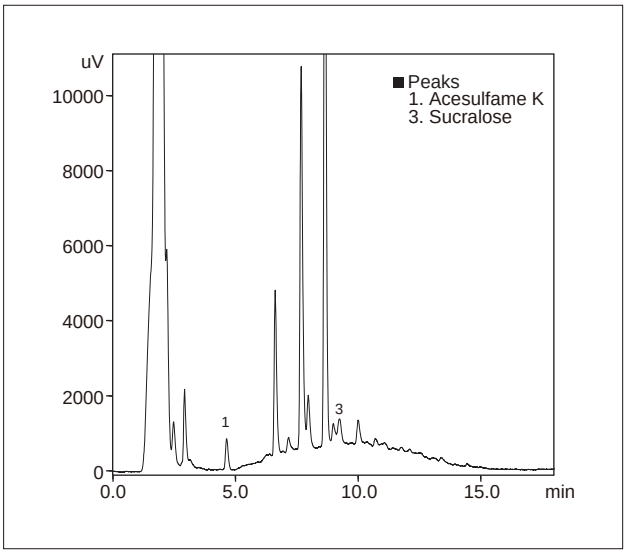


Fig. 2.7.3 Chromatogram of Coffee Drink (10 µL injected)

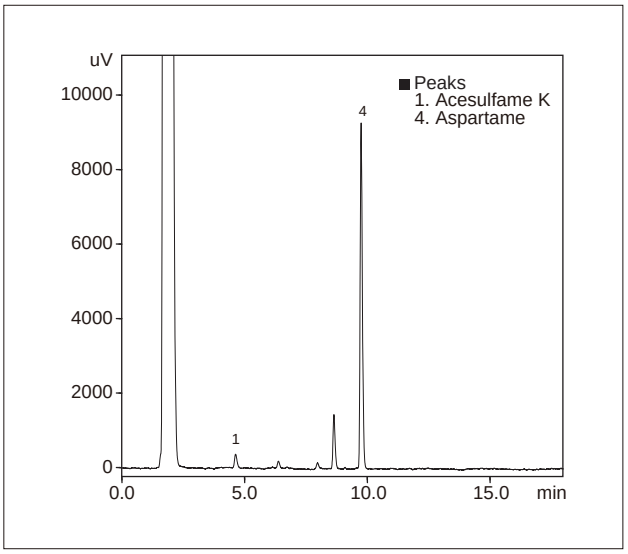


Fig. 2.7.5 Chromatogram of Carbonated Beverage (10 µL injected)

2.8 Analysis of Cyclodextrins in Food (1) - LC

■Explanation

Cyclodextrins are cyclic oligosaccharides consisting of linked D-glucose units in a ring-shaped structure, and it is known that various compounds can be enclosed within that cyclic structure. Properties such as water solubility and stability of the "guest molecules" incorporated into the "host" cyclodextrin can become greatly modified. For this reason they are widely used in the food, pharmaceutical, chemical and other fields. Here we introduce an example of hydrophilic interaction liquid chromatography (HILIC) in which cyclodextrins in a food product are separated, and then detected using the ELSD-LT II Evaporative Light-Scattering Detector.

■Analysis of Standard Solution

In the food industry, α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin are commonly used as food additives. These differ according to the number of linked D-glucose units in the structure, with the α type containing 6 units, the β type 7 units, and the γ type 8 units of linked D-glucose. Fig. 2.8.1 shows the structure of α-cyclodextrin. Here, separation of the cyclodextrins by hydrophilic interaction liquid chromatography (HILIC) was conducted using a polyamine column as the stationary phase, and a mixture of acetonitrile and water as the mobile phase. Detection was conducted using an evaporative light-scattering detector (ELSD). Although detection is also possible using a differential refractive index detector, the ELSD is often very effective in analysis of foods, which typically contain many impurities, because of the applicability of gradient elution methods with the ELSD. Fig. 2.8.2 shows the analysis results of a standard mixture of the 3 cyclodextrins (500 mg/L each).

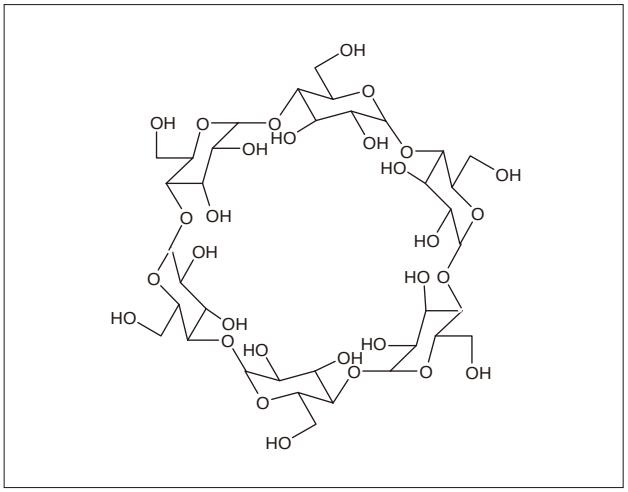


Fig. 2.8.1 Structure of α-Cyclodextrin

■Analytical Conditions

Column	: Asahipak NH2P-50 4E (250 mm L × 4.6 mm I.D.)
Mobile Phase	: 5 mmol/L Ammonium Acetate / Acetonitrile = 35/65 (v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 10 µL
Detection	: ELSD-LT *II*
	Temperature : 40 °C
	Gain : 6
	Nebulizer Gas : N_2
	Gas Pressure : 350 kPa

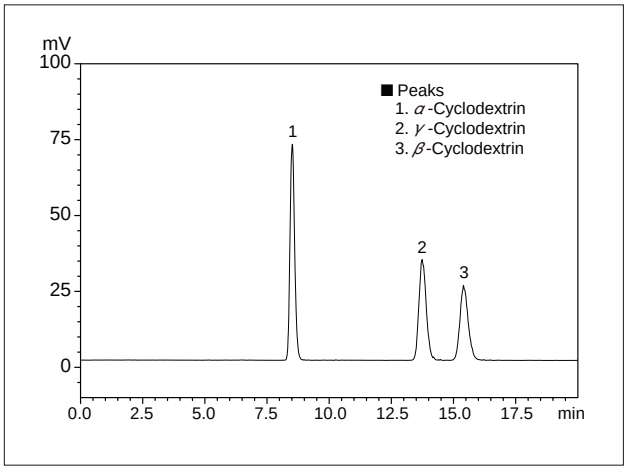


Fig. 2.8.2 Chromatogram of a Standard Mixture of 3 Cyclodextrins (500 mg/L each)

2.8 Analysis of Cyclodextrins in Food (2) - LC

■Analysis of Food Samples

Analyses were conducted for cyclodextrin food additives using a functional drink (isotonic drink), green tea, and Japanese horseradish paste as samples. The hydrophilic impurities contained in these food products were strongly retained on the column specified in the original analytical conditions, so new analytical conditions, including the post-analysis column washing process indicated in this page, were used instead. Fig. 2.8.3 shows the respective sample pretreatment procedures, and Figs. 2.8.4-6 show the chromatograms obtained for each sample.

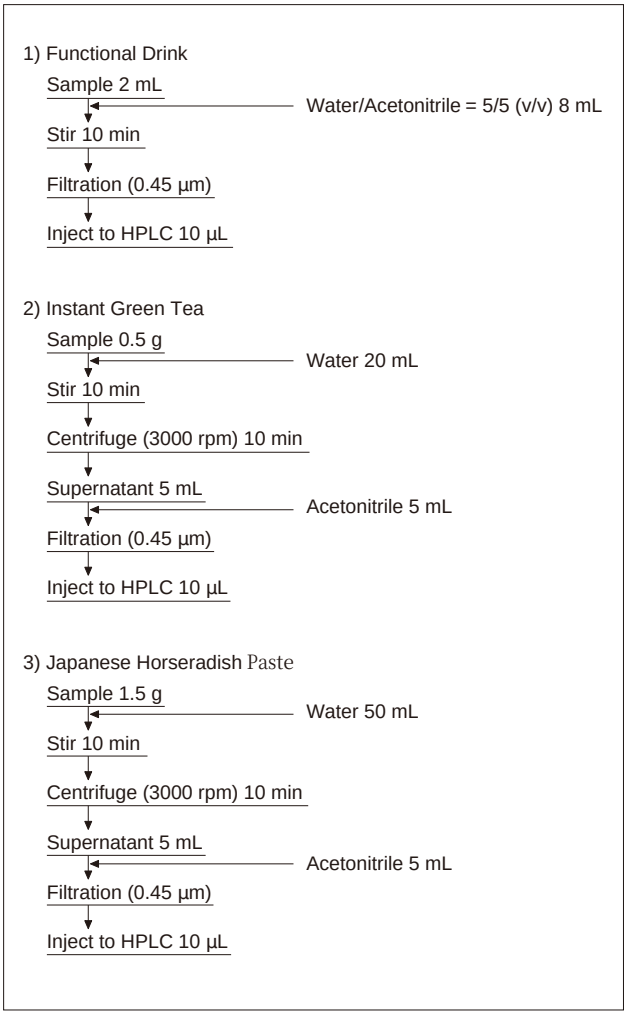


Fig. 2.8.3 Preparation of Samples

■Analytical Conditions

Column	: Asahipak NH2P-50 4E (250 mm L x 4.6 mm I.D.)
Mobile Phase	: A : 5 mmol/L Ammonium Acetate
	B : Acetonitrile
	Gradient Elution Method
Time Program	: B. 65 % (0-17 min) → 45 % (17.01-23 min)
	→ 65 % (23.01-30 min)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 10 µL
Detection	: ELSD-LT II
	Temperature : 40 °C
	Gain : 6
	Nebulizer Gas : N_2
	Gas Pressure : 350 kPa

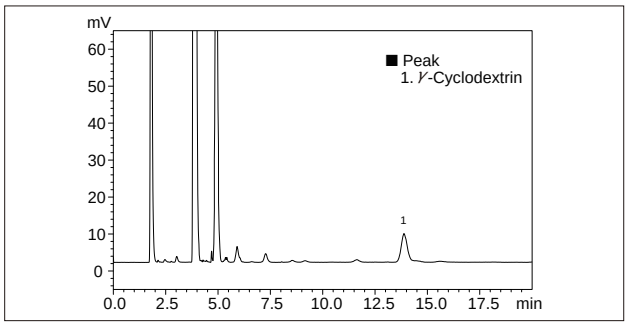


Fig. 2.8.4 Chromatogram of Functional Drink

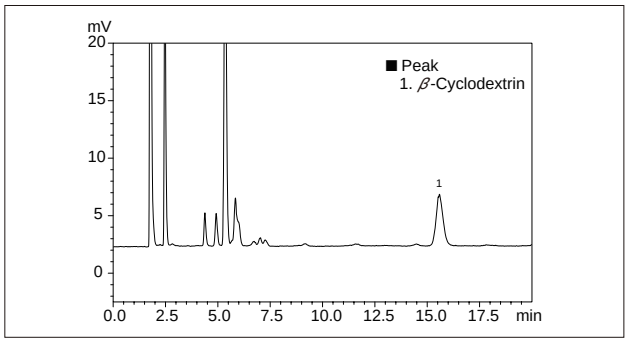


Fig. 2.8.5 Chromatogram of Instant Green Tea

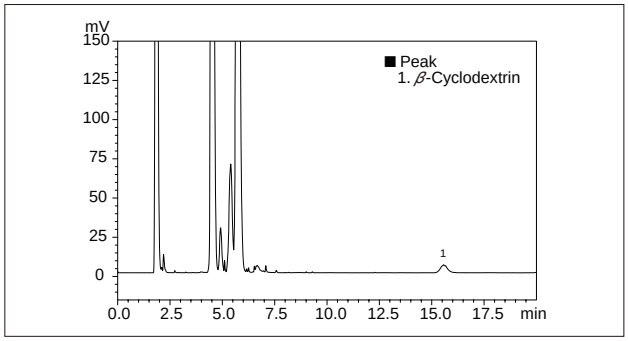


Fig. 2.8.6 Chromatogram of Japanese Horseradish Paste

2.9 Analysis of Sugar Alcohols in Food (1) - LC

■Explanation

Sugar alcohols are widely used in the food industry as low-calorie sweeteners that cause almost no tooth decay. Here we introduce an example of hydrophilic interaction liquid chromatography (HILIC) in which 8 sugar alcohols are separated by gradient elution, and then detected using the ELSD-LT*II* Evaporative Light Scattering Detector.

■Analysis of Standard Solution

Sugar alcohols are chain polyhydric alcohols obtained through reduction of the aldose or ketose carbonyl group. Fig. 2.9.1 shows the structural formulas of 2 typical sugar alcohols, sorbitol and xylitol. Sugar alcohols, as with sugars in general, are substances that have no UV chromophore and while the differential refractive index detector (RID) is generally applicable for detection, it is not very suitable for multi-compound analysis because it cannot be applied in gradient elution analysis. As an alternative, we used an evaporative light scattering detector (ELSD) for simultaneous analysis of 8 sugar alcohols by gradient elution. An aminosilica column was used as the analytical column, and the sugar alcohols were eluted by hydrophilic interaction liquid chromatography (HILIC) using an acetonitrile / water mobile phase. Since the highly hydrophilic impurities that are common in foods are strongly retained on the column, analysis was conducted with a column-washing procedure added to the conditions. Fig. 2.9.2 shows the chromatogram obtained following injection of 10 µL of a standard mixture of 8 sugar alcohols (each 250 mg/L).

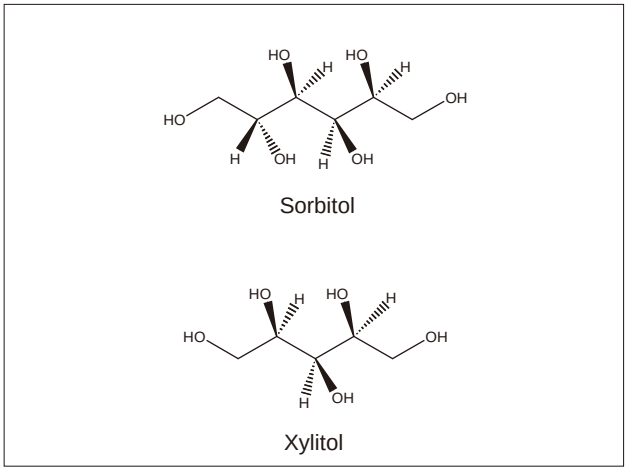


Fig. 2.9.1 Structures of Sorbitol and Xylitol

■Analytical Conditions

Column	: Unison UK-Amino (250 mm L x 4.6 mm I.D. 3 µm)
Mobile Phase	: A : Water B : Acetonitrile Gradient Elution Method
Time Program	: B 90 % (0 min) → 75 % (25 min) → 40 % (25.01-30 min) → 90 % (30.01-40 min)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Detection	: ELSD-LT *II* Temperature : 40 °C Gain : 6 Nebulizer Gas : N_2 Gas Pressure : 350 kPa

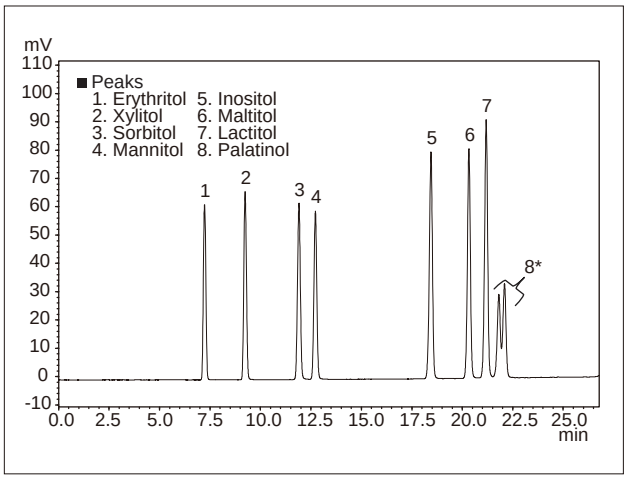


Fig. 2.9.2 Chromatogram of a Standard Mixture of 8 Sugar Alcohols (250 mg/L, 10 µL injected)

*Since Palatinol is a mixture of 1-o-α-D-glucopyranosyl-D-glucitol and 1-o-α-D-glucopyranosyl-D-mannitol, two peaks are eluted.

2.9 Analysis of Sugar Alcohols in Food (2) - LC

Analysis of Food Samples

A functional beverage, a sugarless candy and a sugarless mint tablet were analyzed as food products to which sugar alcohols had been added. Fig. 2.9.3 shows the sample preparation procedure. Erythritol was detected in the functional beverage (Fig. 2.9.4), xylitol, sorbitol and maltitol in the sugarless candy (Fig. 2.9.5), and sorbitol was detected in the sugarless mint tablet (Fig. 2.9.6).

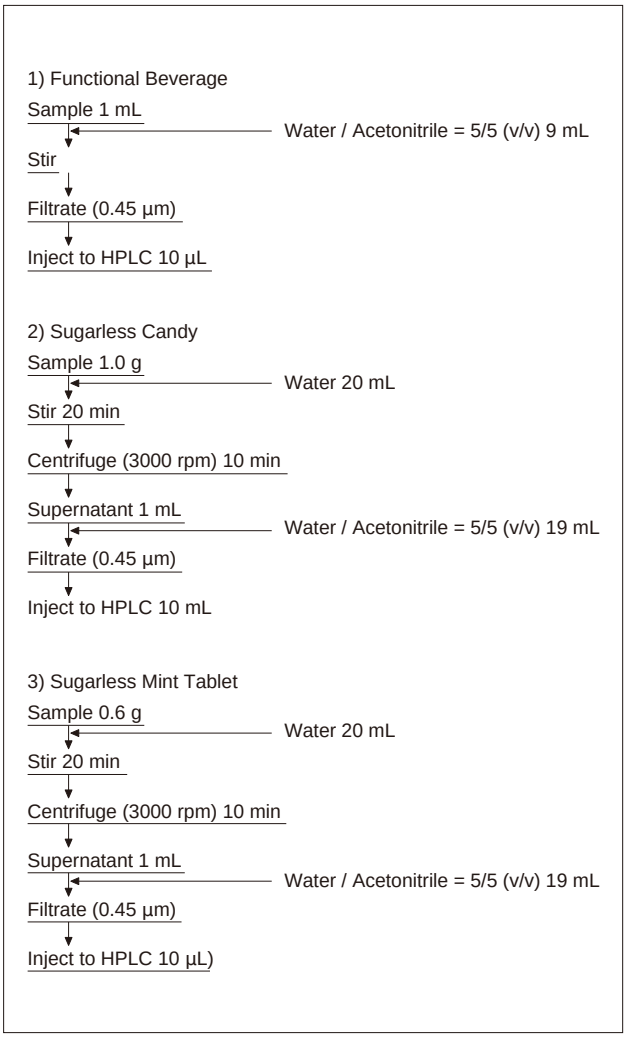


Fig. 2.9.3 Sample Preparation

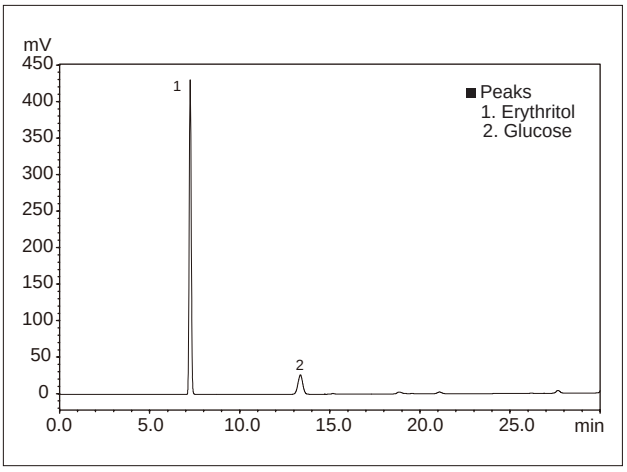


Fig. 2.9.4 Chromatogram of Functional Beverage

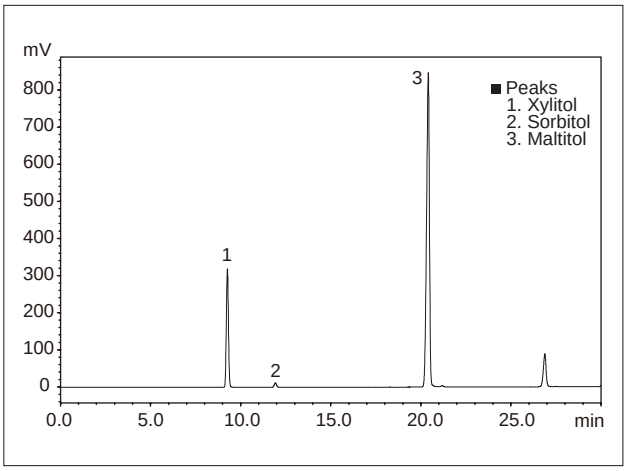


Fig. 2.9.5 Chromatogram of Sugarless Candy

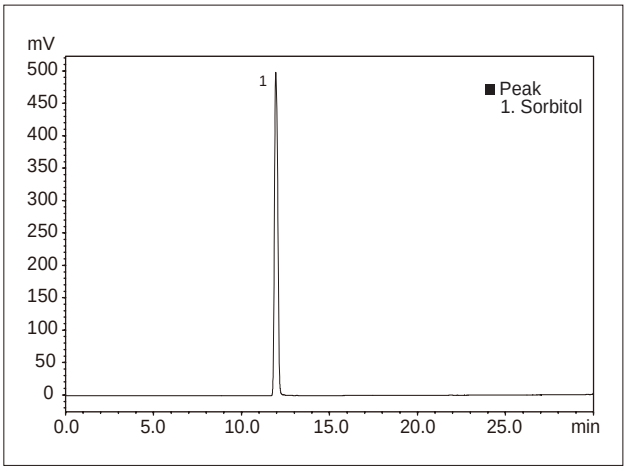


Fig. 2.9.6 Chromatogram of Sugarless Mint Tablet

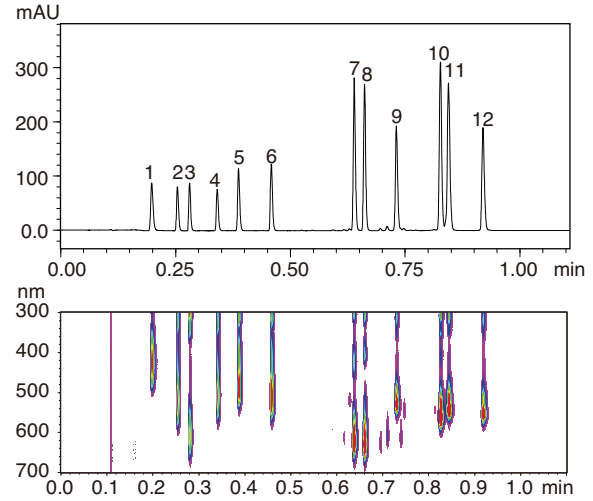
2.10 High Speed Analysis of Artificial Colorings (1) - LC

■Explanation

Here we present an example of ultra-high-speed analysis of artificial colorings using the Nexera UHPLC (Ultra High Performance Liquid Chromatography) System together with the Phenomenex Kinetex C18 high-speed, high-resolution analytical column.

■Analysis of 12 Artificial Colorings

We conducted simultaneous analysis of 12 kinds of tar synthetic dyes. The Phenomenex Kinetex-C18 (particle size 2.6 µm) was used for the separation. This is a Core-Shell column consisting of a 1.9 µm solid core coated with a bonded multilayer (0.35 µm thick) of porous silica gel micro particles. Monitoring was conducted using the maximum absorbance (MAX plot) from 400 to 700 nm with the SPD-M20A photodiode array detector. Fig. 2.10.1 shows the chromatogram of a standard mixture of the 12 artificial colorings (each at 50 mg/L in aqueous solution), in addition to a contour plot. The 12 dyes were separated within 1 minute. In addition, excellent repeatability of retention time and peak area were obtained for all of the components, using 6 consecutive injections (1-µL injections), as shown in Table 2.10.1.



■ Peaks
1. Y4 (Tartrazine), 2. R2 (Amaranth), 3. B2 (Indigo Carmine),
4. R102 (New Coccine), 5. Y5 (Sunset Yellow FCF),
6. R40 (Allura Red AC), 7. G3 (Fast Green FCF),
8. B1 (Brilliant Blue FCF), 9. R3 (Erythrosine), 10. R106 (Acid Red),
11. R104 (Phloxine), 12. R105 (Rose Bengale)

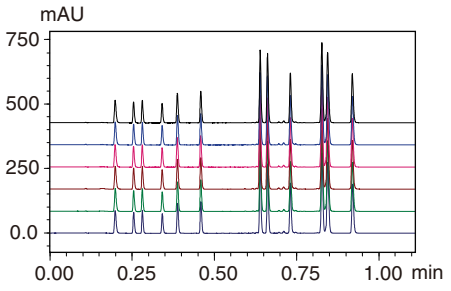
Fig. 2.10.1 Chromatogram and Contour Plot of a Standard Mixture of 12 Artificial Colorings (50 mg/L each)

■Analytical Conditions

Column	: Phenomenex Kinetex 2.6 µm C18 100 Å (50 mm L. × 3.0 mm I.D., 2.6 µm)
Mobile Phase	: A: 10 mmol/L Ammonium Acetate B: 10 mmol/L Ammonium Acetate / Acetonitrile = 1/1 (v/v) Gradient Elution Method
Time Program	: B 5 % (0.0 min) → 100 % (1.1-1.6 min) → 5 % (1.61-2.4 min) • Mixer : 20 µL
Flowrate	: 2.5 mL/min
Column Temp.	: 40 °C
Injection Volume	: 1 µL
Detection	: SPD-M20A Max Plot 400-700 nm
Flow Cell	: Semi-micro cell

Table 2.10.1 Repeatability of 12 Artificial Colorings (n=6)

Peak No.	Retention Time %RSD	Peak Area %RSD
1	0.103	0.117
2	0.069	0.161
3	0.047	0.289
4	0.068	0.219
5	0.091	0.116
6	0.057	0.203
7	0.066	0.170
8	0.056	0.206
9	0.021	0.073
10	0.034	0.117
11	0.032	0.140
12	0.037	0.148



2.10 High Speed Analysis of Artificial Colorings (2) - LC

■Analysis of Artificial Colorings in Food

Pickle juice and liquid extract of candy were measured using the analytical conditions shown on the previous page. The retention times and spectral patterns of the detected peaks matched those of the standard substances.

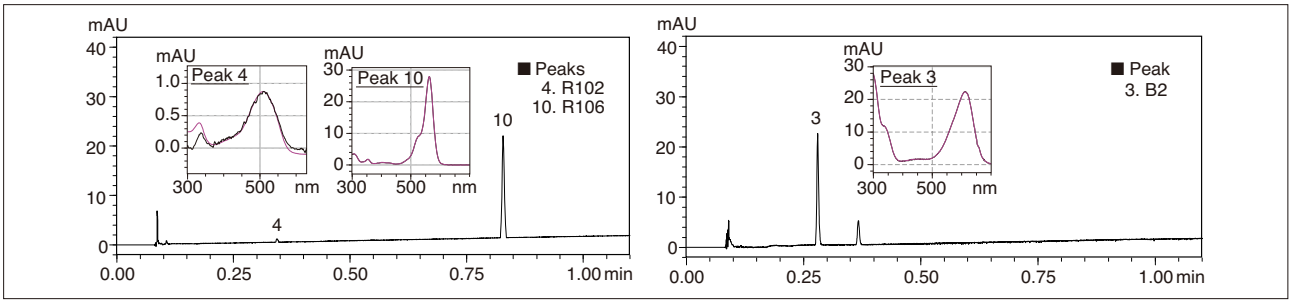


Fig. 2.10.2 Chromatograms and Spectra of Artificial Colorings in Food (Left: Pickle, Right: Candy)

■Analysis of 21 Artificial Colorings

We conducted analysis of 21 artificial substances, consisting of those listed on the previous page together with 9 additional dye substances. Based on investigation of the gradient analysis conditions (Table 2.10.2), simultaneous analysis was completed within 2 minutes. Fig. 2.10.3 shows the MAX plot and the chromatograms obtained using 3 different wavelengths. Chromatographic peaks were identified for yellow dyes at 450 nm, red dyes at 520 nm, and blue-green dyes at 620 nm. Thus, simultaneous quantitation can be achieved by selecting the appropriate detection wavelength for each substance using the photodiode array detector. In the MAX plot, peak 20 (Orange *II*) and peak 21 (Patent Blue V) are overlapping, however, as shown in Fig. 2.10.4, at 450 nm and 620 nm, almost none of the other substances are present, allowing quantitation at each separate wavelength.

Table 2.10.2 Analytical Conditions

Time Program	: B 5 % (0.0 min) → 100 % (2.5-2.7 min) → 5 % (2.71-3.5 min)
Detection	: SPD-M20A Max Plot 400-700 nm, 450 nm, 520 nm, 620 nm

*The other conditions are the same as specified on the previous page.

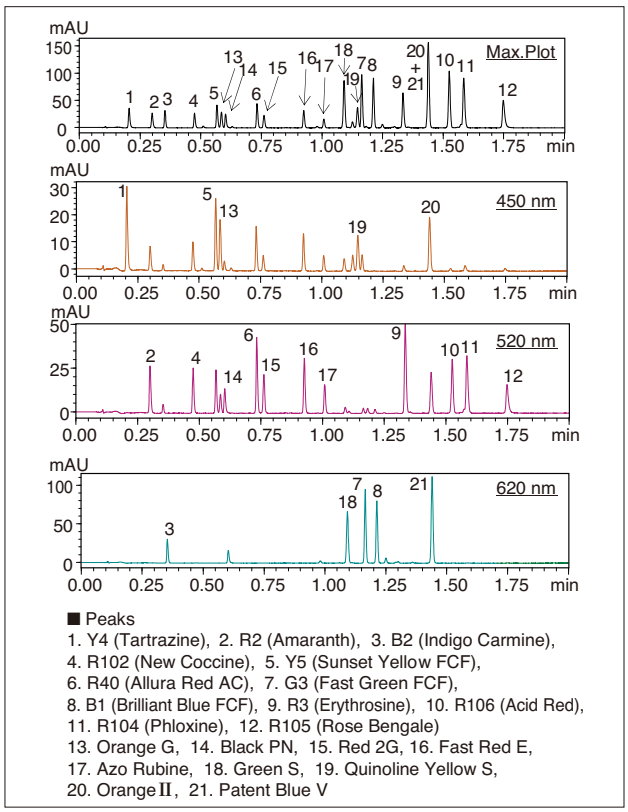


Fig. 2.10.3 Multi-Chromatogram of a Standard Mixture of 21 Artificial Colorings (25 mg/L each)

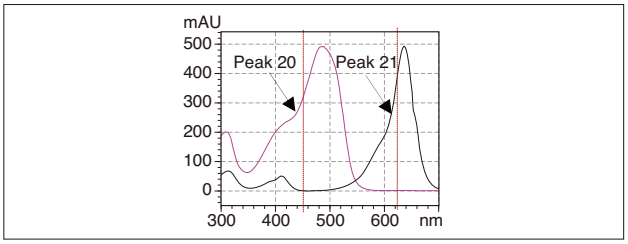


Fig. 2.10.4 Spectra of Peak 20 and Peak 21

2.11 Analysis of Curcumin in Turmeric - LC

■Explanation

Analysis of herbal medicines by HPLC generally requires separation of the impurities and active constituents, so the time to required to complete an analysis becomes relatively long. Here we introduce examples of high-speed analysis of herbal medicines using the "Prominence UFLC" ultra-fast LC system with the "Shim-pack XR-ODS" high-speed, high-resolution column.

■Analysis of Curcumin in Turmeric

Curcumin, which is present in turmeric, is used not only as an artificial yellow coloring agent, but it is also effective for enhancing liver function and promoting bile secretion. The sample preparation procedure is shown in Fig. 2.11.1. Fig. 2.11.2 shows an analysis example of curcumin in turmeric.

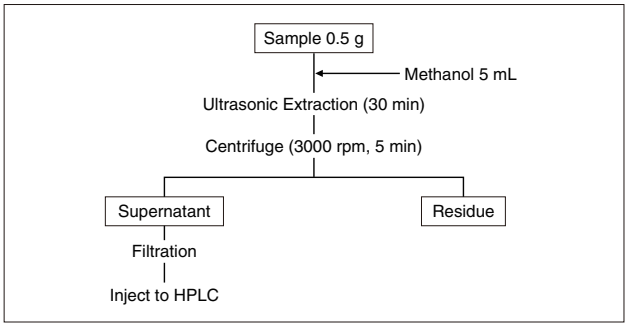


Fig. 2.11.1 Sample Preparation

■Analytical Conditions

Column	: Shim-pack XR-ODS (75 mm L. × 3.0 mm I.D., 2.2 μm)
Mobile Phase	: A: 2 % Acetic Acid aq. B: Acetonitrile A / B = 55/45 (v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 4 μL
Detection	: SPD-20AV at 425 nm
Flow Cell	: Semi-micro Cell

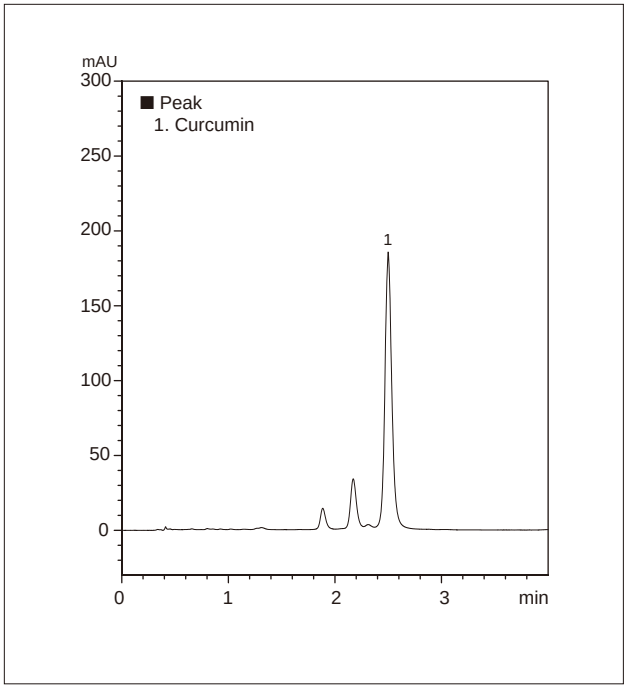


Fig. 2.11.2 Chromatogram of Turmeric

2.12 Analysis of Impurities in Curcumin (1) - LC/MS

■Explanation

Here we present an example of analysis of a curcumin using photodiode array (PDA) detection and mass spectrometry (MS).

■Analysis of Curcumin Standard Solutions

Curcumin is a type of polyphenol present in turmeric, and is used as a food dye. Fig. 2.12.1 shows the structrures of curcumin in addition to two similar compounds (curcuminoids). The measurement samples consisted of commercial curcumin standards of different purity, referred to as grade A and grade B. The standard solutions were prepared by dissolving the standards in methanol and adjusting the respective concentrations to 1 mg/mL. PDA detection was conducted at a wavelength of 425 nm. MS analysis was conducted using electrospray ionization, and the deprotonated molecules were detected. Grade A showed a relatively low purity of curcumin, and more than 20 minutes was required to complete the analysis using a 5 µm particle diameter column (Fig. 2.12.2 (a), (b)), while this analysis time was shortened by 7 minutes using the Prominence UFLC (Fig. 2.12.2 (c), (d)). The area percentages in the PDA chromatogram were 1.11 % for bisdemethoxycurcumin, 11.46 % for demethoxycurcumin, and 87.19 % for curcumin. Other than these compounds, minute peaks were also noticeable at t_R=1.34 min (λ max 338 nm, *m/z* 337), t_R=1.48 min (λ max 349 nm, *m/z* 367), and t_R=2.77 min (λ max 429 nm, *m/z* 383). As for the highpurity grade B sample, neither bisdemethoxycurcumin nor demethoxycurcumin were detected (Fig. 2.12.2 (e), (f)).

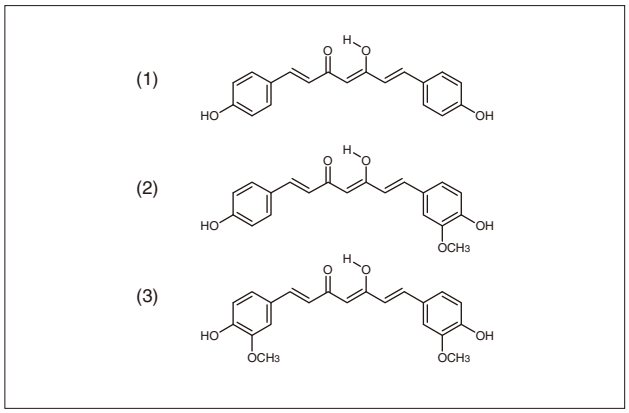


Fig. 2.12.1 Structures of Curcuminoids
(1) Bisdemethoxycurcumin (MW 308),
(2) Demethoxycurcumin (MW 338),
(3) Curcumin (MW 368)

■Analytical Conditions

Column	: Shim-pack XR-ODS (75 mm L. × 2.0 mm I.D., 2.2 µm, UFLC) Shim-pack VP-ODS (150 mm L. × 2.0 mm I.D., 5 µm, HPLC)
Mobile Phase	: 0.1 % Formic Acid / Acetonitrile = 60/40
Flowrate	: 0.4 mL/min (UFLC), 0.2 mL/min (HPLC)
Column Temp.	: 40 °C
Injection volume	: 1 µL
Detection	
PDA	
SPD-M20A	: 425 nm
MS	
Probe Voltage	: -3.5 kV (ESI-Negative Mode)
Nebulizing Gas Flow	: 1.5 L/min
Drying Gas Pressure	: 0.15 MPa (UFLC), 0.10 MPa (HPLC)
CDL Temp.	: 250 °C
Block Heater Temp.	: 200 °C
CDL, Q-array Voltages	: Using default values
Scan Range	: *m/z* 150 - 500

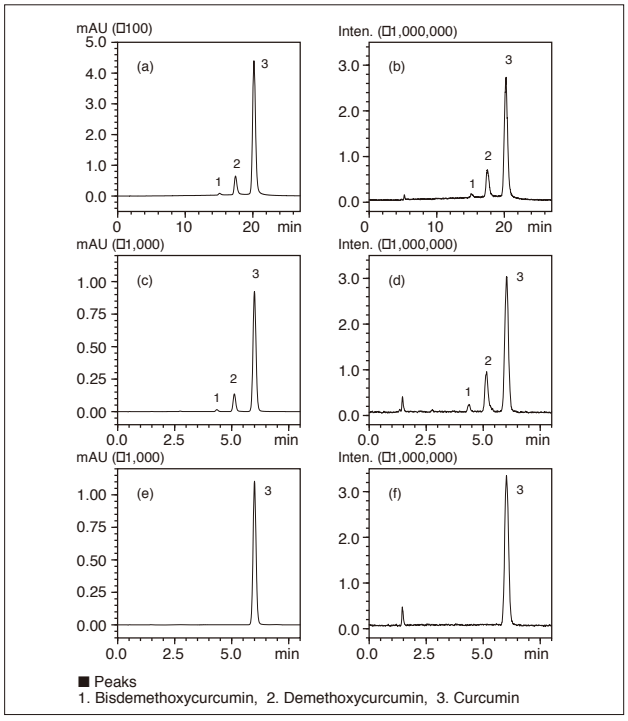


Fig. 2.12.2 PDA Chromatograms, Total Ion Chromatograms (TIC) of Curcumin Standard Solutions
(a) PDA chromatogram of curcumin (grade A) by HPLC,
(b) TIC of curcumin (grade A) by HPLC-MS
(c) PDA chromatogram of curcumin (grade A) by UFLC
(d) TIC of curcumin (grade A) by UFLC-MS
(e) PDA chromatogram of curcumin (grade B) by UFLC
(f) TIC of curcumin (grade B) by UFLC-MS

2.12 Analysis of Impurities in Curcumin (2) - LC/MS

■PDA Data

Fig. 2.12.3 shows the UV spectra of curcuminoids. All of them clearly possess a maximum absorption wavelength near 420 nm. When the peak purity is calculated from the grade A data, an impurity was detected at 5.35 minutes (Fig. 2.12.4 (2)).

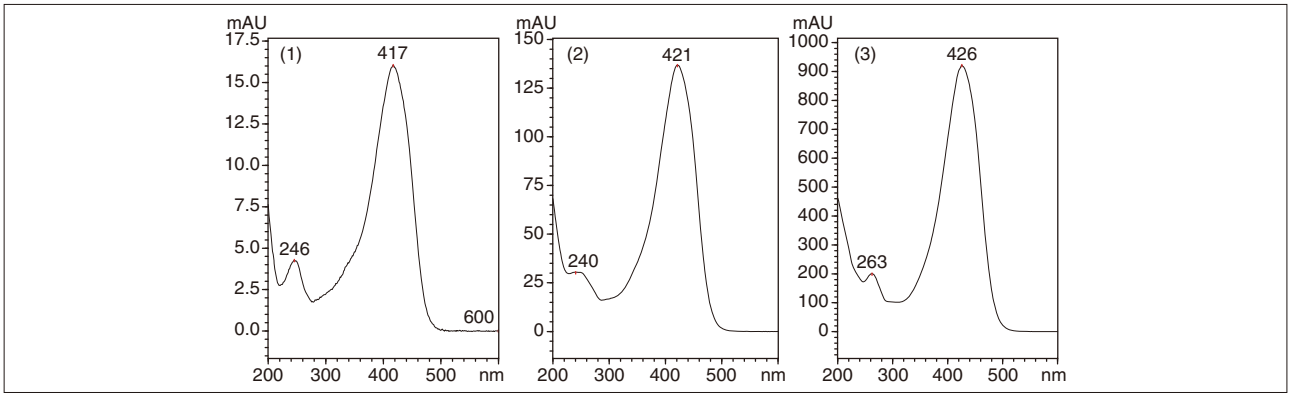


Fig. 2.12.3 UV Spectra of Curcuminoids ((1) Bisdemethoxycurcumin, (2) Demethoxycurcumin, (3) Curcumin)

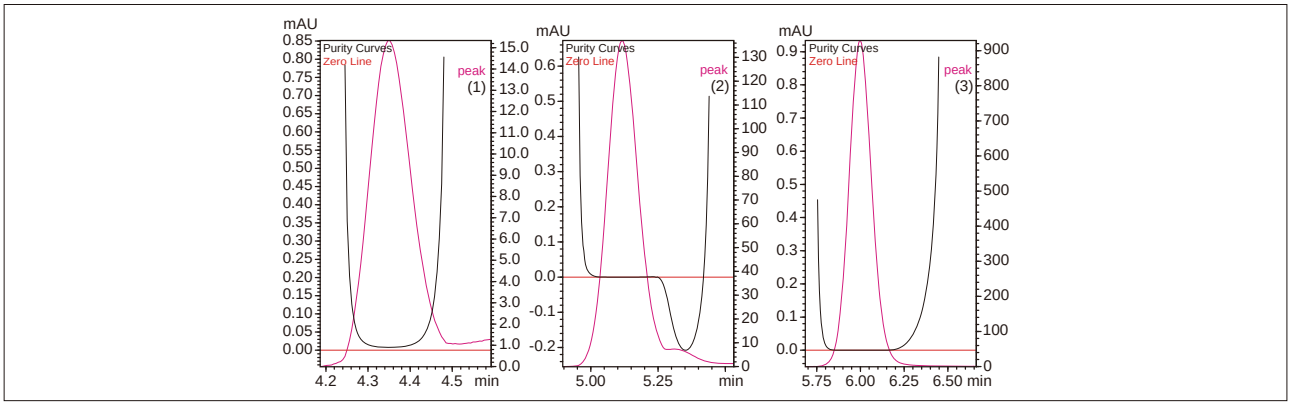


Fig. 2.12.4 Purity Curves of Curcuminoids ((1) Bisdemethoxycurcumin, (2) Demethoxycurcumin, (3) Curcumin)

■Another Impurity

Focusing on the peak (compound X) at 5.35 minutes in the mass chromatograms of Fig. 2.12.4 (2), the UV spectrum and mass spectrum were obtained for this peak, as shown in the Fig. 2.12.5. The maximum absorption wavelength is in the vicinity of 370 nm, and the *m/z* 369 negative ion was detected. Therefore, the molecular weight of compound X is presumed to 370. Thus, by using both PDA and MS in UFLC detection, greater efficiency is believed to be possible.

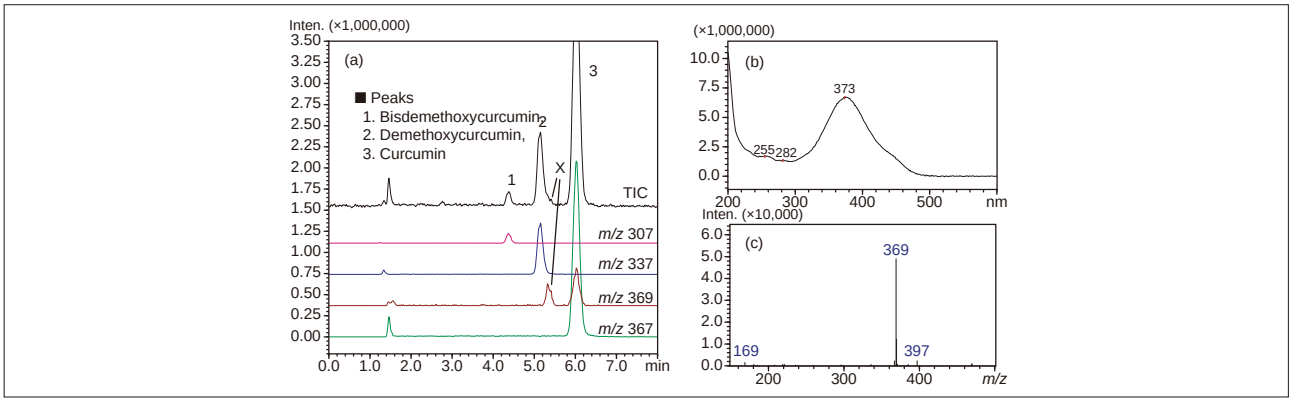


Fig. 2.12.5 Mass chromatograms of curcumin standard solution (grade A, (a)) and UV spectrum (b), mass spectrum (c) of compound X

2.13 Analysis of Sudan Dyes in Foods - LC

■Explanation

Sudan dyes are oil-soluble red synthetic dyes that are used in industrial products. These dyes are forbidden to be added to food products in Japan, Europe and the United States, however, since Sudan *I* was detected in imported cayenne in Europe in May 2003, the presence of Sudan *I* in foods have been reported in Europe and other places. Monitoring inspections for Sudan dyes are being reinforced in Japan, as well. Sudanese dyes include Sudan *I* , Sudan *II* , Sudan *III* , and Sudan *IV*. Sudan *IV* has also been found in Europe and other places. Here we introduce simultaneous HPLC analysis of seven dyes, including Sudan *I*-*IV*, Sudan Orange G, Sudan Red G and Sudan Red 7B.

■Analysis of Standard Solution

Fig. 2.13.1 shows the chromatogram of a 10 µL injection of a standard mixture of Sudan dyes (each concentration was 100 mg/L, which was obtained by 10-fold dilution of 1 g/L of acetone solution with ethanol.).

* Since Sudan Orange G was confirmed as two isolated peaks, they are indicated as Sudan Orange G-a and Sudan Orange G-b respectively.

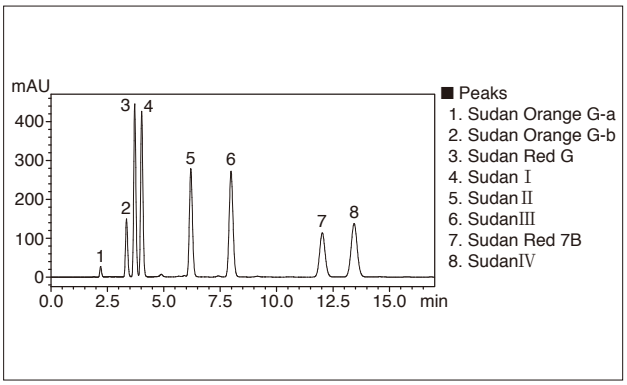


Fig. 2.13.1 Chromatogram of a Standard Mixture of Sudan Dyes (100 mg/L each, 10 µL inj.)

■Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D.)
Mobile Phase	: 0.1 % (v/v) Formic Acid aq. / Acetonitrile = 1/9 (v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 10 µL
Detection	: SPD-20AV at 480 nm

■Analysis of Curry Powder

Fig. 2.13.3 shows the chromatogram of commercial curry powder spiked with standard samples of Sudan Dyes. Seven types of Sudan dyes were added to the curry powder to make 100 µg/g for each dye, and after pretreatment as shown in Fig. 2.13.2, 10 µL of the sample solution was injected into the HPLC.

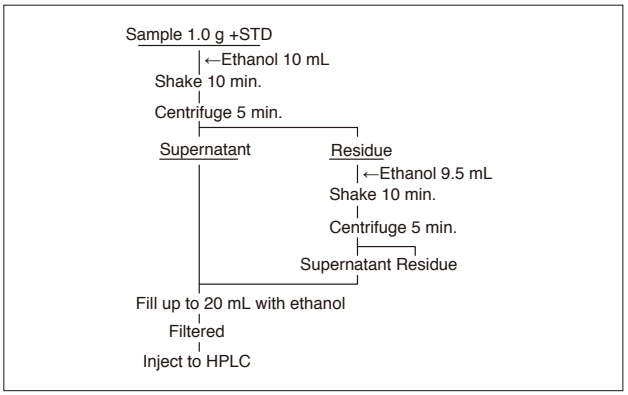


Fig. 2.13.2 Sample Pretreatment

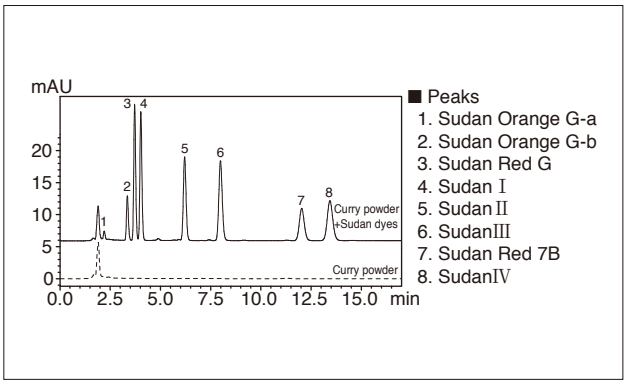


Fig. 2.13.3 Chromatogram of Curry Powder Spiked with Sudan Dye Standards (each added at 100 µg/g)

2.14 Analysis of Sudan Dyes and Para Red - LC/MS

■Explanation

Sudan dyes are red or orange synthetic pigments used to color various dyeing oils and waxes, etc. Sudan dyes are not permitted in food in many countries, including Japan, the EU and the United States, due to concerns that they may be carcinogenic. However, there are some countries where these dyes are still being used for coloring of such spices as chili powders and paprika. Since Sudan *I* was first detected in cayenne products imported into the EU in May 2003, there have been many reported cases of its detection, and this has led to strengthened inspection of such products when they are imported into Japan. The Ministry of Health, Labour and Welfare Administration of Food Safety Publication No.0501008, "Test Methods for Sudan Dyes and Para Red in Foods" (hereafter referred to as "official methods"), dated May 1, 2006, specifies the use of HPLC as the quantitation method, and LC/MS as one of the confirmation test methods. Here we introduce an example of simultaneous quantitative analysis of Sudan dyes and Para Red using LC/MS. Fig. 2.14.1 shows the structural formulas and mass spectra for Sudan *I*-*IV* and Para Red. Fig. 2.14.2 shows the SIM chromatograms obtained for Sudan *I*-*IV* and Para Red added to commercial chili powder.

■Analytical Conditions

Column	: Shim-pack FC-ODS (2.0 mm I.D. × 150 mm)
Mobile Phase	: Water containing 0.1 % Formic Acid / Acetonitrile = 15/85
Flowrate	: 0.2 mL/min
Column Temp.	: 40 °C
Injection Volume	: 1 µL
Probe Voltage	: 4.5 kV (ESI-Positive Mode)
CDL Temp.	: 250 °C
BH Temp.	: 200 °C
Nebulizing Gas Flow	: 1.5 L/min
Drying Gas Pressure	: 0.10 MPa
CDL Voltage	: Using default value
Q-array DC Voltage	: Using default value
Q-array RF Voltage	: Using default value
Scan Range	: *m/z* 90-500
SIM Monitoring Ions	: *m/z* 294, 249, 277, 353, 381

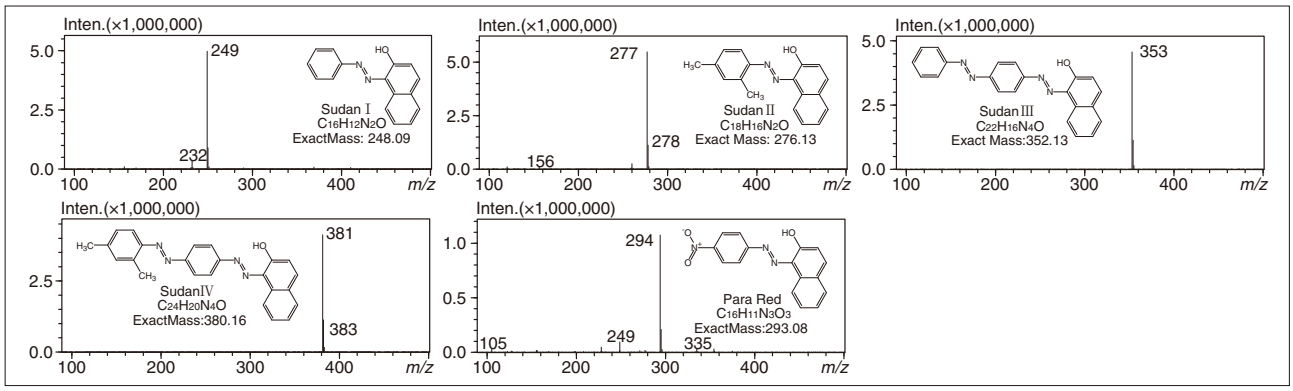


Fig. 2.14.1 Positive ESI Mass Spectra of Sudan Dyes and Para Red

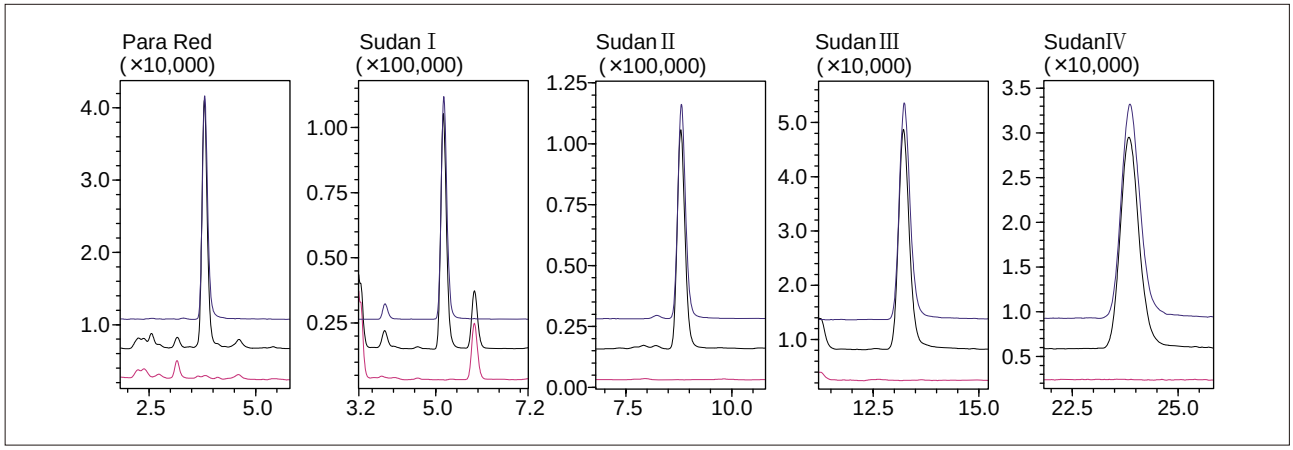


Fig. 2.14.2 SIM Chromatograms of Chili Powder Extract with 5 µg/mL Sudan Dyes and Para Red (Black), Chili Powder Extract (Red), and Sudan Dyes and Para Red (Blue) at 500 ng/mL each

2.15 Analysis of Essential Oil - GC

■Explanation

Here, direct GC analysis examples of peppermint oil and spearmint oil used as flavorings are introduced.

■Analytical Conditions

Column	: ULBON HR-20M (50 m × 0.25 mm I.D. df = 0.25 μm)
Column Temp.	: 60 °C -3 °C/min - 220 °C
Injector Temp.	: 250 °C
Detector Temp.	: 250 °C (FID)
Carrier Gas	: He (1.4 mL/min)
Injection Method	: Split Injection
Split Ratio	: 1:15
Injection Volume	: 0.2 μL

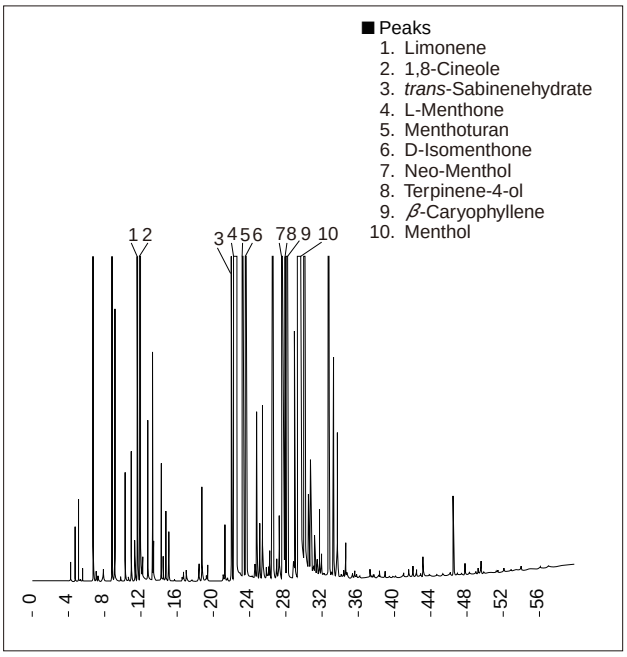


Fig. 2.15.1 Analysis of Peppermint Oil

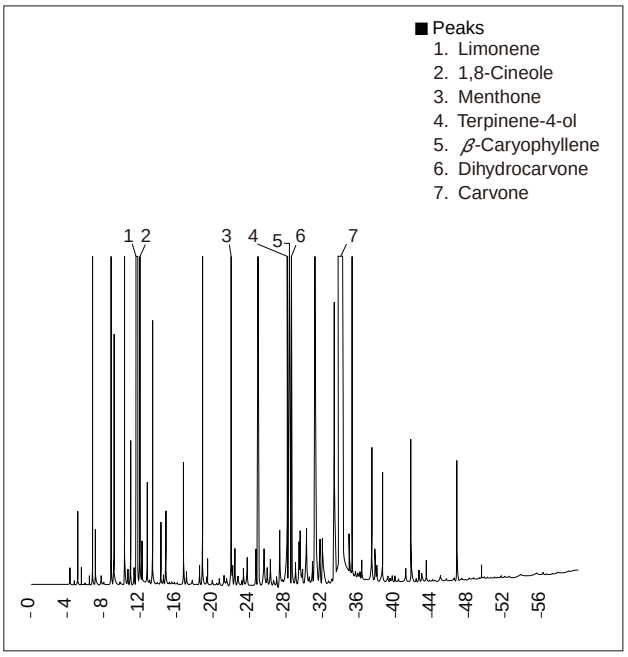


Fig. 2.15.2 Analysis of Spearmint Oil

2.16 Flavor Analysis by Fast - GC/MS (1) - GC/MS

■Explanation

Fast-GC/MS is an effective way to improve laboratory productivity by shortening analysis cycle times. Fast-GC/MS methods require a system with a high-performance AFC controller that is compatible with narrow bore capillary columns and high-speed data acquisition technology. Shimadzu GCMS systems offer the high-performance levels necessary to satisfy these requirements. Here we show the results from analyzing lavender oil using conventional methods and Fast-GC/MS methods.

■Analytical Conditions

Conventional-GC/MS

Instrument	: GCMS-QP2010 Ultra
Column	: Rtx-5MS (30 mL. × 0.32 mm I.D., 0.25 μm)
Glass Insert	: Split insert with deactivated glass wool (P/N: 225-20803-01)

[GC]		[MS]	
Injection Temp.	: 250 °C	Interface Temp.	: 250 °C
Column Temp.	: 50 °C (0 min) - (3 °C /min) - 250 °C (10 min)	Ion Source Temp.	: 200 °C
		Measurement Mode	: Scan
Injection Mode	: Split	Mass Range	: *m/z* 40 - 400
Carrier Gas	: He	Event Time	: 0.3 sec
Control Mode	: Linear velocity (47.2 cm/sec)	Emission Current	: 150 μA (high sensitivity)
Split Ratio	: 1:100		
Injection Volume	: 2.0 μL		

Fast-GC/MS

Instrument	: GCMS-QP2010 Ultra
Column	: Rtx-5 (10 mL.× 0.10 mm I.D., 0.1 μm)
Glass Insert	: Split insert with deactivated glass wool (P/N: 225-20803-01)

[GC]		[MS]	
Injection Temp.	: 250 °C	Interface Temp.	: 250 °C
Column Temp.	: 70 °C (1 min) - (25 °C /min) - 180 °C - (50 °C /min) - 280 °C (1 min)	Ion Source Temp.	: 200 °C
		Measurement Mode	: Scan
Injection Mode	: Split	Mass Range	: *m/z* 40 - 400
Carrier Gas	: He	Event Time	: 0.05 sec
Control Mode	: Linear velocity (45.0 cm/sec)	Emission Current	: 150 μA (high sensitivity)
Split Ratio	: 1:1800		
Injection Volume	: 1.0 μL		

2.16 Flavor Analysis by Fast - GC/MS (2) - GC/MS

■Results

Fast-GC/MS provided separation patterns similar to results from conventional methods, but analysis times were 1/7 of conventional analysis times.

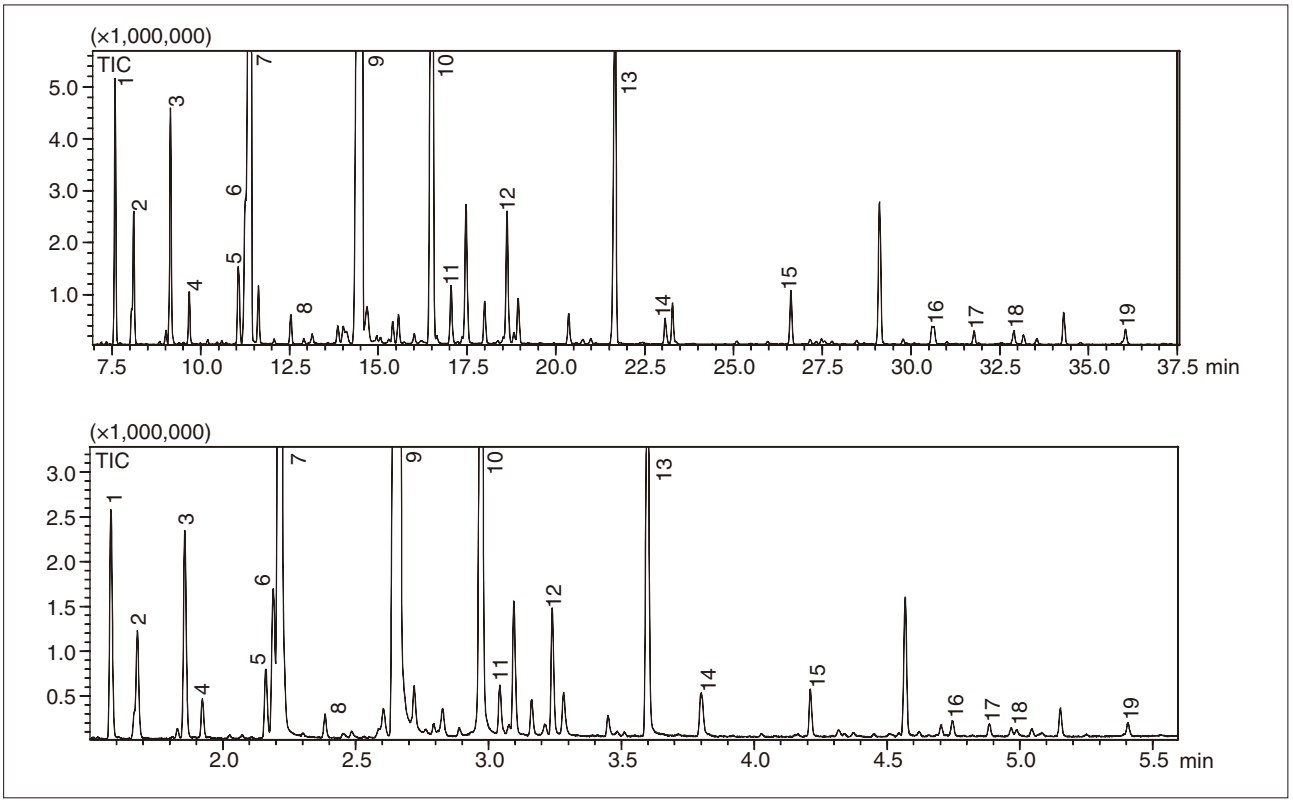


Fig. 2.16.1 Total Ion Current Chromatograms Obtained Using Conventional Method (upper) and Fast-GC/MS Method (lower)

ID	Compound Name	ID	Compound Name	ID	Compound Name
1	alpha-Pinene	8	gamma-Terpinene	14	Bornyl acetate
2	Camphene	9	Linalool	15	Neryl acetate
3	beta-Pinene	10	Camphor	16	(Z)-beta-Farnesene
4	Myrcene	11	Isoborneol	17	Germacrene D
5	Cymene	12	alpha-Terpineol	18	beta-Bisabolene
6	Limonene	13	Linalyl acetate	19	Caryophyllene oxide
7	Eucalyptol				

2.17 Analysis of Benzoyl Peroxide in Foods - LC

■Explanation

In Japan, the food additive benzoyl peroxide is permitted to be used as a processing agent only in flour, and its concentration is limited to 0.30 g/kg or less in the "Specifications and Standards of Foods and Food Additives" published by the Japanese Ministry of Health, Labour and Welfare. In the Food Safety Standards Notification No. 0513003 (May 13, 2004), the benzoyl peroxide analysis method was changed from gas chromatography to HPLC. Here we introduce an example of benzoyl peroxide analysis based on the method specified in the Food Safety Standards Notification No. 0513003.

■Analytical Conditions

Column	: Shim-pack VP-ODS (250 mm L. × 4.6 mm I.D.)
Mobile Phase	: Water / Acetonitrile = 45/55 (v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Detection	: UV235 nm

■Analysis of Flour

Fig. 2.17.2 shows the sample preparation procedure for analyzing benzoyl peroxide in flour. Fig. 2.17.3 shows the chromatograms obtained by analyzing a sample of flour produced in Japan (after preparation), and a sample spiked with 1.0 mg/L benzoyl peroxide (equivalent to 5.0 mg/kg in flour).

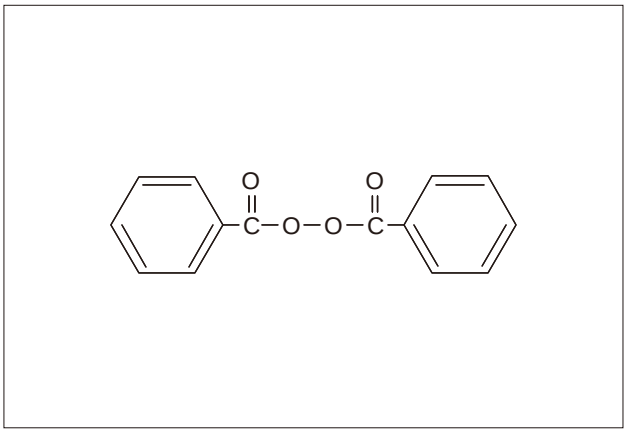


Fig. 2.17.1 Structure of Benzoyl Peroxide

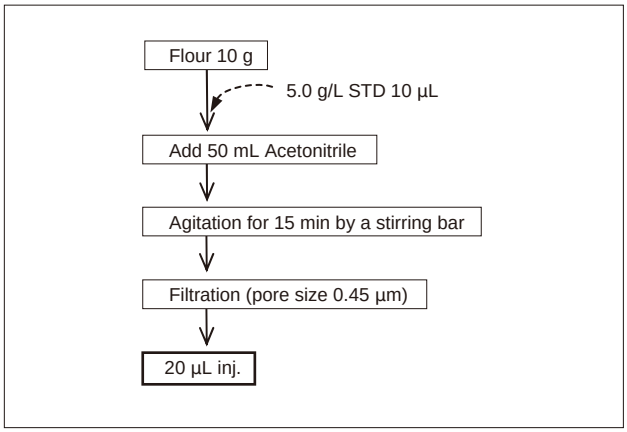


Fig. 2.17.2 Sample Preparation

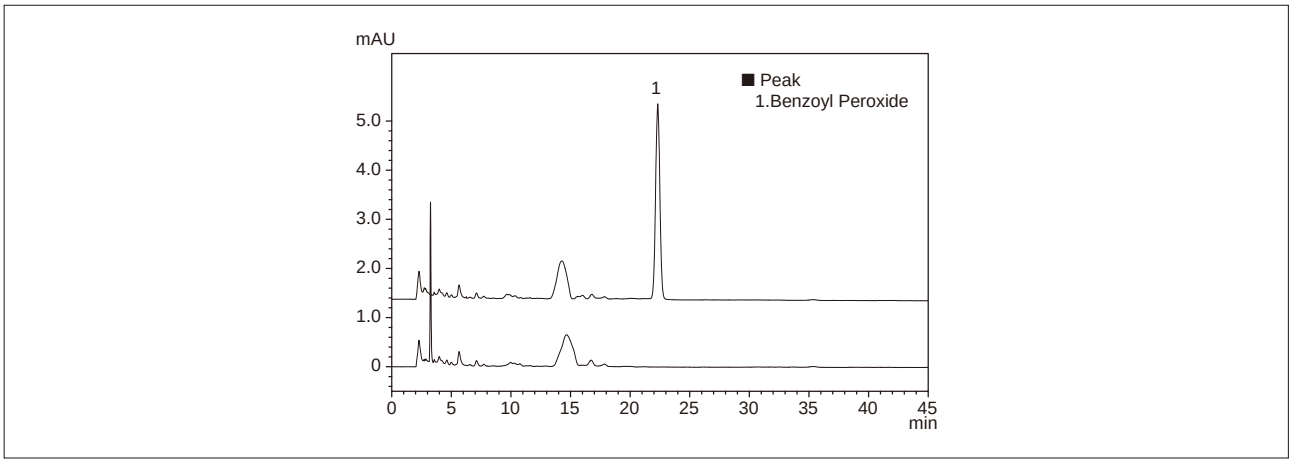


Fig. 2.17.3 Chromatogram of Flour sample - Upper: Spiked with 1.0 mg/L Benzoyl Peroxide, Lower: Not spiked (20 µL inj. each)

3. Aromas and Odors

3.1 Analysis of Aroma Compounds in Peach Juice (1) - GC/MS

■Explanation

The OPTIC-4 is a multimode injection system with a thermal desorption function that enables performing thermal desorption by placing an adsorbent in the injection port liner cup. In combination with the new MonoTrap adsorbent, the system enables analyzing aroma components with high sensitivity.

The MonoTrap is a completely new trapping tool with a proprietary monolithic porous high-purity silica structure that concentrates trace components using the adsorptive capacity provided by its large surface area. Due to a wide variety of adsorbent materials used in the MonoTrap, it can efficiently concentrate many compounds, including polar compounds, enabling ultra-high-sensitivity analysis. This was used as a monolithic material sorptive extraction (MMSE) method for GC/MS pretreatment. In this case, the MonoTrap RGC18TD (for thermal desorption), a hybrid graphite carbon and octadecyl group type sorbent, was used to concentrate aroma components in peach juice, which were analyzed using a high-polarity capillary column.

■Experimental

Collection

30 mL of commercial peach juice and MonoTrap RGC18 TD were placed in a 40 mL vial and stirred for one hour at room temperature for sampling.

Analysis

The MonoTrap material was removed from the vial, its surface lightly rinsed with water, and placed in a specialized liner, then placed in the OPTIC-4 inlet for thermal desorption.

MonoTrap RGC18 TD
Cat. No.1050-74201
(Manufactured by GL Sciences Inc.)

Specialized Liner
Cat. No.1003 -75001
(Manufactured by GL Sciences Inc.)

■Analytical Conditions

Instruments

Injection (TD) : OPTIC-4 (ATAS GL International BV, Eindhoven, the Netherlands)
GC-MS : GCMS-QP2010 Ultra (Shimadzu)
Column : InertCap Pure-WAX (0.25 mm × 30 m, df = 0.25 μm (GL Sciences, Japan.)

[Injector]

Thermal Desorption Temp.	: 40° C - (50 °C/sec) - 200 °C
Carrier Gas	: He
Column Flowrate	: 1.0 mL/min
Split Flowrate	: 5:50
Cryofocus Temp.	: Trap -160 °C Introduction 250 °C

[MS]

Interface Temp.	: 250 °C
Ion Source Temp.	: 200 °C
Solvent Elution Time	: 0.5 min
Data Sampling Time	: 1.0 - 60 min
Measurement Mode	: TIC
Mass Range	: *m/z* 30-600
Detector Voltage	: +0.87 kV (absolute value)

[GC]

Column Temp. : 40 °C (5 min) - (4 °C /min) - 250 °C (5 min)

3.1 Analysis of Aroma Compounds in Peach Juice (2) - GC/MS

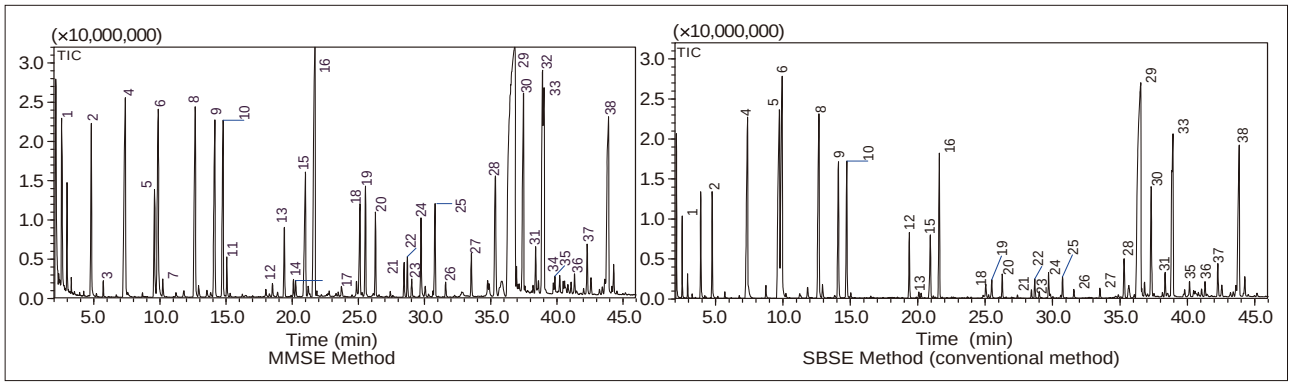


Fig. 3.1.1 Comparison of Chromatograms Obtained Using the MMSE (with MonoTrap) and Conventional (SBSE) Methods

1. Ethyl acetate
2. Ethyl butanoate
3. Butyl acetate
4. Isoamyl acetate
5. D-Limonene
6. Isobutyl isovalerate
7. 2-Hexenal
8. Hexyl acetate
9. 3-Hexenyl acetate
10. 2-Hexenyl acetate
11. 2-Isopropyl-4-methylthiazole
12. Octyl acetate
13. Benzaldehyde
- 2-Methyl-4-propyl-1,3-oxathiane
- *p*-Menthan-2-one
16. beta-Linalool
17. 2-Methylbutanoic acid
18. gamma-Caprolactone
19. Terpineol
20. Benzyl acetate
21. *cis*-Geraniol
22. beta-Damascenone
- 5-Methyl-2- (1-methyl-1-sulfanylethyl) cyclohexanone
- *trans*-Geraniol
25. gamma-Butylbutyrolactone
26. beta-Ionone
27. gamma-*n*-Amylbutyrolactone
28. Triacetin
29. delta-Undecalactone
30. delta-Decalactone
31. Eudesm-7 (11) -en-4-ol
32. Ethyl caproate
33. delta-Undecalactone
34. *n*-Decanoic acid
35. delta-Hexylvalerolactone
36. gamma-Dodecalactone
37. delta-Dodecalactone
38. Nootkatone

In Fig. 3.1.2, the peak area values obtained for each component by the SBSE method are indicated as "1" to show how MMSE sensitivity compares to SBSE. Because the MonoTrap RGC18 TD material is a hybrid of graphite carbon and octadecyl groups, it is more effective in collecting polar compounds than the SBSE method, which uses agitator paddles coated with PDMS. Therefore, the MonoTrap material enabled highly sensitive analysis. It is especially useful for sulfur compounds and lactones, such as gamma-caprolactone (18) and 2-Isopropyl-4-methylthiazole (11).

Lactones are the components in peaches, other fruits, dairy products, etc., that give them a sweet smell. 2-Isopropyl-4-methylthiazole is used as a fruit and vegetable flavoring.

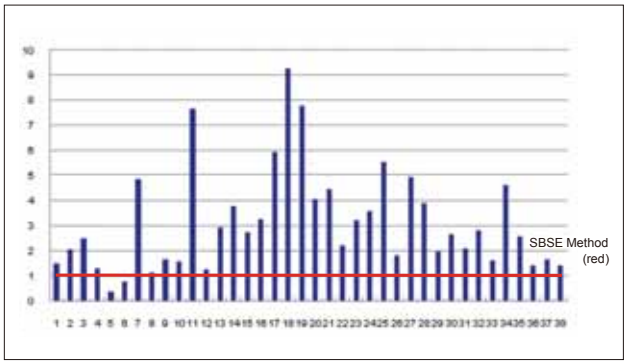


Fig. 3.1.2 Sensitivity Comparison Between MMSE and Conventional (SBSE) Methods

The monolith manufacturing technology with sol-gel method is new technology developed in Japan by Dr. Soga and Dr. Nakanishi at Kyoto University.

H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N.Tanaka, Anal. Chem. 1996, 68, 3498-3501.

Nakanishi, K., Pore structure control of silica gels based on phase separation. *J. Porous Materials*, 4 (1997) 67

■Summary

The MMSE method using the MonoTrap monolithic adsorbent enabled the use of a simple procedure to highly concentrate trace aroma components. The high sensitivity is not only due to the high concentration levels, but also due to the improved adsorption efficiency of the graphite carbon contained in the MonoTrap's base silica structure and to the use of a high-polarity deactivated column. In addition, the improved sensitivity for polar compounds, which had previously been an issue when analyzing aroma components, was presumably due to the efficient thermal desorption provided by the OPTIC-4 multimode inlet. The combination of concentration by the MMSE method and the OPTIC-4 multimode inlet provides an excellent alternative to SBSE and other typical concentrating methods for detecting or screening low-concentration compounds in gaseous form.

3.2 Analysis of Aroma Compounds in Cheese (1) - GC/MS

■Explanation

Volatile compounds, including aroma compounds, in Parmesan and Blue cheese were analyzed using adsorption and thermal desorption GC/MS (TD-GC/MS). MonoTrap was used as adsorbant. It is a state-of-the-art silica monolithic and hybrid adsorbent having a large surface area and properties based on silica, activated carbon (graphite carbon for Mono Trap TD) and the octadecyl functional group. GC-MS equipped with the OPTIC-4 multi-purpose injector was used for thermal desorption. The OPTIC-4 allows direct introduction of desorbed gas into a capillary column without re-adsorption.

■Experimental

Sample Preparation

MonoTrap RGC18 TD is conditioned and packed into an ampoule (Fig. 3.2.1) before shipment and therefore showed an extremely low blank. It was used without conditioning. Ten grams of each cheese sample were weighed and placed into a vial (40 mL). MonoTrap was placed over the sample in the vial using the MonoTrap holder. The vials were capped and agitated for 3 hours at 600 °C.

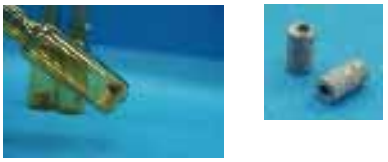


Fig. 3.2.1 MonoTrap Packed into an Ampoule for TD (left)
MonoTrap RGC18 TD (right)

TD-GC/MS Analysis

MonoTrap RGC18 TD was removed and placed into the OPTIC-4 liner.

Fig. 3.2.2 OPTIC-4 (left) and GCMS-QP2010 Ultra equipped with OPTIC-4 and AOC-5000 Plus (right). Liner can be automatically exchanged using the AOC-5000 Plus.

■Analytical Conditions

Instruments
Injection Unit : OPTIC-4
GC-MS : GCMS-QP2010 Ultra
Auto-Sampler : AOC-5000 Plus LINEX system
Column : InertCap Pure-WAX (60 m × 0.25 mm I.D., df = 0.25 µm (GL Sciences, Inc.)

[OPTIC-4]
Desorb Temp. : 200 ºC
Desorb Time : 5 min
Carrier Gas : He
Column Flow : 1.0 mL/min
Injection Mode : Splitless
Cryo Trapping : -150 ºC
Injection Temp. : 250 ºC

[MS]
Interface Temp. : 230 ºC
Ion Source Temp. : 200 ºC
Acquisition Mode : Scan
Mass Range : *m/z* 29-600

[GC]
Column Temp. : 40 ºC (5 min) - (6 ºC /min) - 250 °C

3.2 Analysis of Aroma Compounds in Cheese (2) - GC/MS

■Results and Discussion

Fig. 3.2.3 and Fig. 3.2.4 show total ion current chromatograms of Parmesan and Blue cheese, respectively. The detected compounds were identified using a mass spectral library search. Sulfur compounds, such as dimethyl disulfide and dimethyl sulfone, were extracted and detected. It is known to be difficult to detect sulfur compounds using conventional TD system.

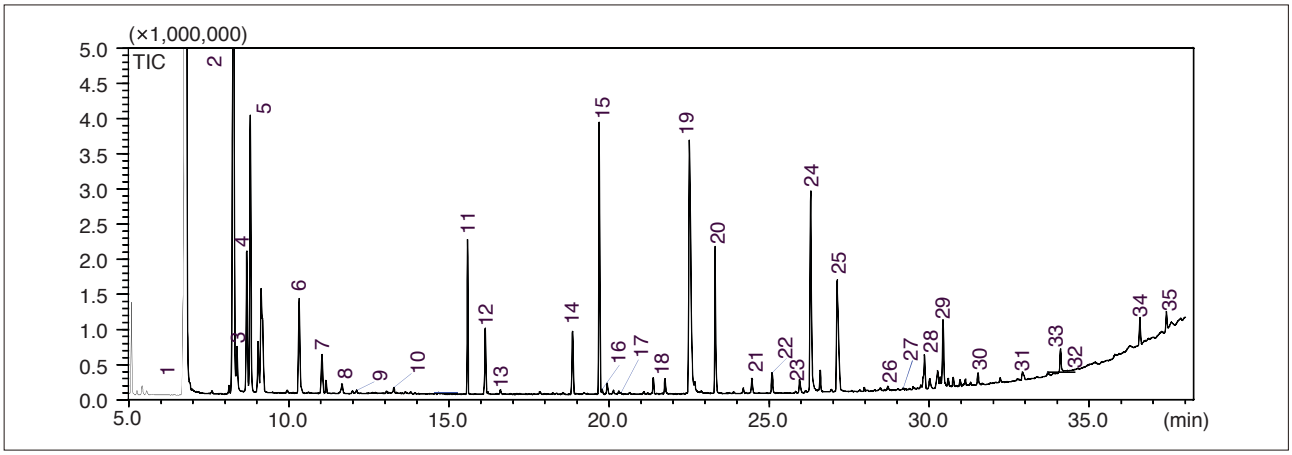


Fig. 3.2.3 Total Ion Current Chromatogram of Parmesan Cheese

1. Methanethiol, 2. Ethyl acetate, 3. 2-Butanone, 4. 2-Methylbutanal, 5. 3-Methylbutanal, 6. 1-Propanol, 7. Toluene, 8. Dimethyl disulfide, 9. Hexanal, 10. 2-Pentenal, 11. 3-Penten-2-one, 12. 2-Heptanone, 13. D-Limonene, 14. Acetoin, 15. Acetol, 16. Dimethylpyrazine, 17. Dimethylpyrazine, 18. Dimethylpyrazine, 19. 2-Nonanone, 20. 2, 5-Dimethyl-3-ethylpyrazine, 21. Benzaldehyde, 22. Isobutyric acid, 23. 2-Undecanone, 24. Butanoic acid, 25. 2-Furanmethanol, 26. Acetamide, 27. 2-Tetradecanol, 28. 2-Tridecanone, 29. Hexanoic acid, 30. Dimethyl sulfone, 31. δ-Octalactone, 32. 2-Pentadecanone, 33. Octanoic acid, 34. δ-Decalactone, 35. Decanoic acid

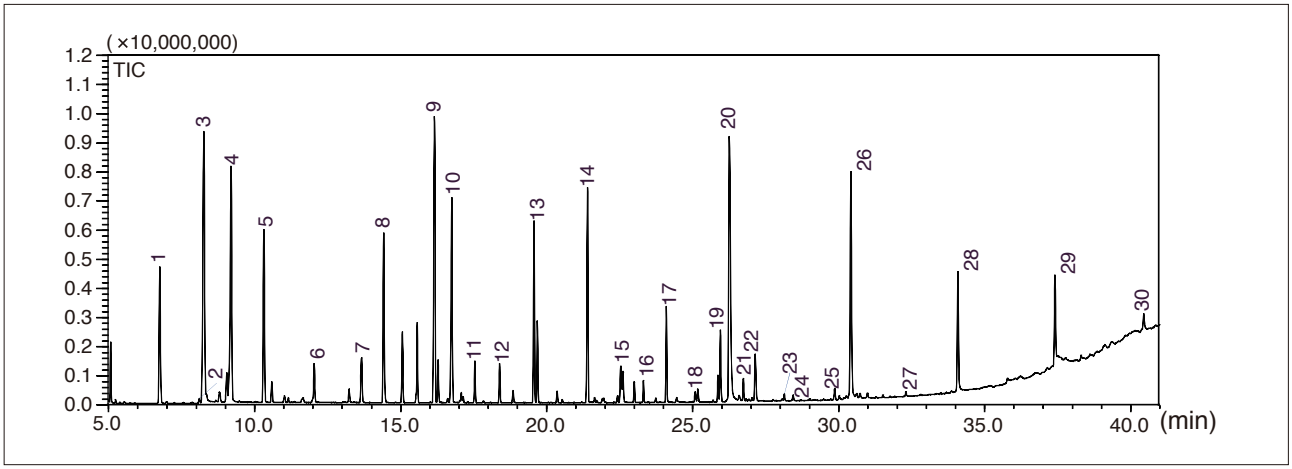


Fig. 3.2.4 Total Ion Current Chromatogram of Blue Cheese

1. Acetaldehyde, 2. Butanal, 3. Ethyl acetate, 4. Isovaleraldehyde, 5. 2-Pentanone, 6. Ethyl butyrate, 7. 2-Hexanone, 8. Isobutyl alcohol, 9. 2-Heptanone, 10. Methylhexanoate, 11. Ethylhexanoate, 12. 1-Pentanol, 13. 2-Heptanol, 14. 2-Nonanone, 15. Ethyloctanoate, 16. 2-Decanone, 17. 2-Nonanol, 18. Methyldecanoate, 19. 2-Undecanone, 20. Butanoic acid, 21. Ethyldecanoate, 22. 3-Methylbutanote, 23. γ-Caprolactone, 24. 2-Undecanol, 25. 2-Tridecanone, 26. Hexanoic acid, 27. 2-Pentadecanone, 28. Octanoic acid, 29. Decanoic acid, 30. Dodecanoic acid

■Summary

Sulfur compounds in the cheese were easily and simply detected using MonoTrap and TD-GC/MS (OPTIC-4 and GC-MS).

3.3 Higher Sensitivity Analysis of 2-Methoxy-3-Isobutylpyrazine (MIBP) in Wine (1) - GC/MS/MS

■Explanation

2-Methoxy-3-isobutylpyrazine (MIBP) is an aromatic substance with the fragrance of bell peppers. It is found in sauvignon blanc (a type of grape used for white wine) and cabernet sauvignon (a type of grape used for red wine), and gives the wines a favorable aroma. MIBP, which has a significant impact on the flavor of wine, has an extremely low threshold value in sensory tests, on the order of a few ng/L. Since wine contains many components, concentration and selective separation and detection are essential to analysis.

The trace amounts of MIBP in wine were selectively detected by utilizing the MonoTrap® silica monolithic absorbent for collection and concentration, and a GC/MS/MS (GCMS-TQ8030) in positive chemical ionization (PCI) mode. The MRM acquisition mode was used to monitor specific transitions for the compound of interest.

■Experimental

A standard MIBP solution was added to commercially-available sauvignon blanc (produced in Chile in 2012) and cabernet sauvignon (produced in Chile in 2012) at different concentrations (0 ng/L, 1 ng/L, 5 ng/L, 10 ng/L, and 20 ng/L). The samples were heated for 1 hour at 50 °C, and then the gaseous phase MIBP was collected using MonoTrap® RGPS TDNote (GL Sciences, P/N: 1050-74202). After collection, the MonoTrap® RGPS TD was measured using the following analytical conditions.

■Analytical Conditions

Autosampler	: AOC-5000 Plus
OPTIC Liner Auto Exchange System	: CDC + LINEX
Multipurpose Injection Port	: OPTIC-4
GC-MS	: GCMS-TQ8030
Column	: InertCap 17MS (Length: 30 m, 0.25 mm I.D., df = 0.25 µm), (GL Sciences, P/N: 1010-20142)

[OPTIC-4]

Initial Temp.	: 35 ºC
Ramp Rate	: 10 ºC/sec
Hold Temp.	: 250 ºC
Hold Time	: 300 sec
Column Flow1	: 5 mL/min
Column Flow Time	: 320 sec
Column Flow2	: 1.5 mL/min
Split Flow1	: 5 mL/min
Split Flow Time	: 320 sec
Split Flow2	: 50 mL/min

[GC]

Column Temp.	: 40 ºC (5 min) - (10 ºC/min) - 280 ºC (5 min)

[MS]

Ion Source Temp.	: 200 ºC
Interface Temp.	: 250 ºC
Ionization Method	: Positive chemical ionization (PCI)
Reagent Gas	: Isobutane (60 kPa)
Acquisition Mode	: MRM
Event Time	: 0.3 sec
Monitor Ion and Collision Energy (CE)	: *m/z* 167.1 > 124.1 (20 V) *m/z* 167.1 > 135.1 (15 V)

■Results

Fig. 3.3.1 shows the calibrations curves for sauvignon blanc and cabernet sauvignon via the standard addition method. Favorable results were obtained for the correlation coefficient (R) between area and concentration for each MIBP-spiked sample, with 0.9999 for the sauvignon blanc and 0.9998 for the cabernet sauvignon. Fig. 3.3.2 shows the MRM chromatograms for the MIBP in the wines. Table 3.3.1 shows the results of quantitatively analyzing each wine 3 times via the standard addition method and the repeatability. The respective concentrations of MIBP in the wines were 5.4 ng/L for the sauvignon blanc and 12.1 ng/L for the cabernet sauvignon. In addition, favorable results of 3 % RSD were obtained for the repeatability.

3.3 Higher Sensitivity Analysis of 2-Methoxy-3-Isobutylpyrazine (MIBP) in Wine (2) - GC/MS/MS

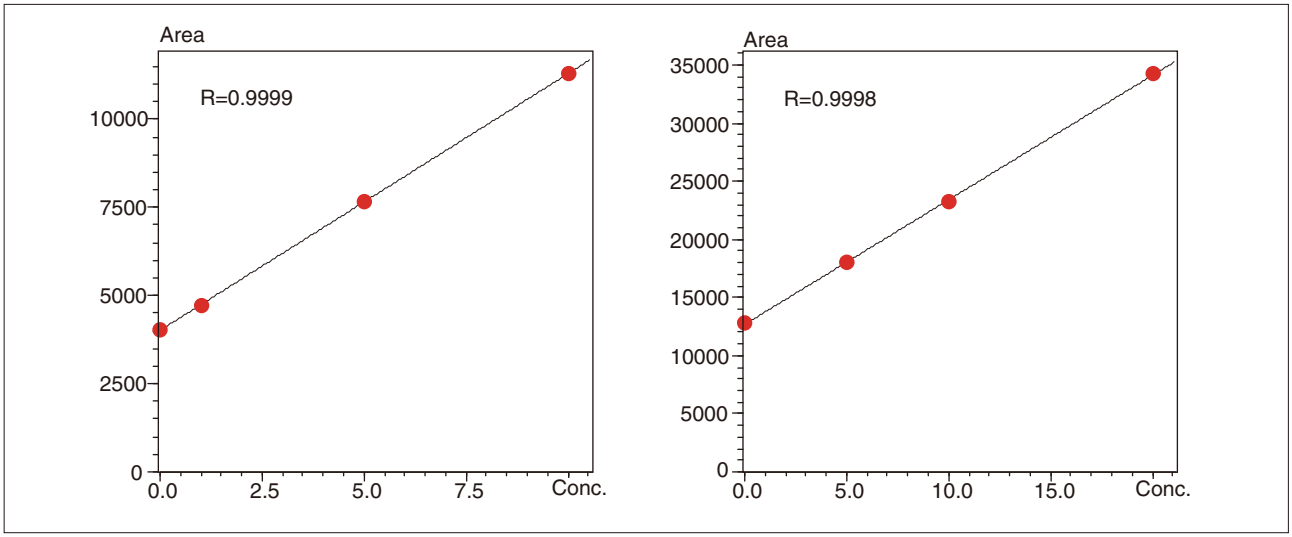


Fig. 3.3.1 Calibration Curves for the Wines via the Standard Addition Method
Left: Sauvignon Blanc (Concentrations of 0 ng/L, 1 ng/L, 5 ng/L, and 10 ng/L)
Right: Cabernet Sauvignon (Concentrations of 0 ng/L, 5 ng/L, 10 ng/L, and 20 ng/L)

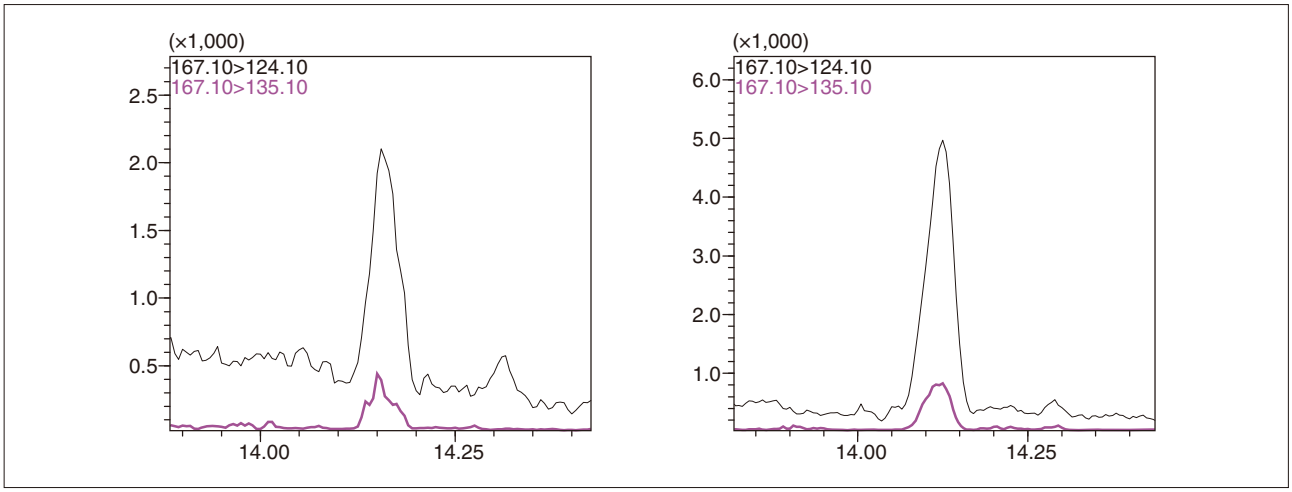


Fig. 3.3.2 MRM Chromatograms for the MIBP in the Wines (Left: Sauvignon Blanc, Right: Cabernet Sauvignon)

Table 3.3.1 Quantitative Results for the MIBP in the Wines via the Standard Addition Method (Concentration Units: ng/L), and the Repeatability (n=3)

Wine Type	1	2	3	Average	Standard Deviation	C.V. (%)
Sauvignon Blanc	5.5	5.3	5.3	5.4	0.1	2.47
Cabernet Sauvignon	11.8	12.3	12.1	12.1	0.2	1.91

■Conclusion

The trace quantities of MIBP in the wines were collected and concentrated by the MonoTrap® RGPS TD, and then selectively detected by utilizing the GC-MS/MS in PCI mode with MRM acquisition mode. It was thus possible to detect MIBP at the ng/L level with high sensitivity.

4. Residual Pesticides

4.1 Analysis of Organophosphorus Pesticides in Baby Foods (1) - GC/MS/MS

Introduction

Contamination of food products with pesticides is a growing global concern, particularly for baby foods. GC/MS/MS operated in the Multiple Reaction Monitoring (MRM) mode is a technique of choice for analysis of trace contaminants in complex matrices because of its improved sensitivity and selectivity compared to single quadrupole GCMS.

Experimental

Samples were prepared using the QuEChERS (Quick Easy Cheap Effective Rugged and Safe) sample preparation method[1]. Analyses were conducted using a Shimadzu GCMS-TQ8030 GC/MS/MS operated in the MRM mode using conditions detailed Shimadzu Application News GCMS-1304[2]. A 7-point calibration curve was prepared for 24 OP-pesticides using the matrix-matched internal standard procedure, and a sample of organic blended peas to minimize contribution of analytes from the sample matrix.

Results and Discussion

GCMSMS Operation in the MRM Mode

Operation of the GCMS-TQ8030 in the MRM mode provides excellent sensitivity and selectivity for analysis of trace level contaminants in complex matrices. Relationship between the responses of the pesticides of interest relative to the matrix background is shown in Fig. 4.1.1.

The selectivity of the MRM mode is illustrated in the chromatograms of three OP pesticides shown in Fig. 4.1.2. In Fig. 4.1.2, the top chromatograms are the extracted ion chromatograms (mass chromatograms) from the full-scan (Q3 scan) data, and the bottom chromatograms are the corresponding chromatograms from the MRM mode. The MRM operation mode allows detection and quantitation of trace concentrations of analytes in complex matrices such as food extracts, virtually eliminating background interference.

Calibration Results and Assessment of Precision

Seven calibration standards were prepared in the blended peas extract over the range of 0.5-200 ng/mL (ppb). Response factors were calculated and relative standard deviation (RSD) determined by the GCMSsolution software. The precision of the calibration was evaluated using the RSD of the response factors and the correlation coefficient (r) for each of the analytes. In general, the % RSD was < 25 % and correlation coefficient values for the multi-point calibration were > 0.999.

Example calibration curves are shown in Fig. 4.1.3; chromatograms correspond to the 1 ppb standard.

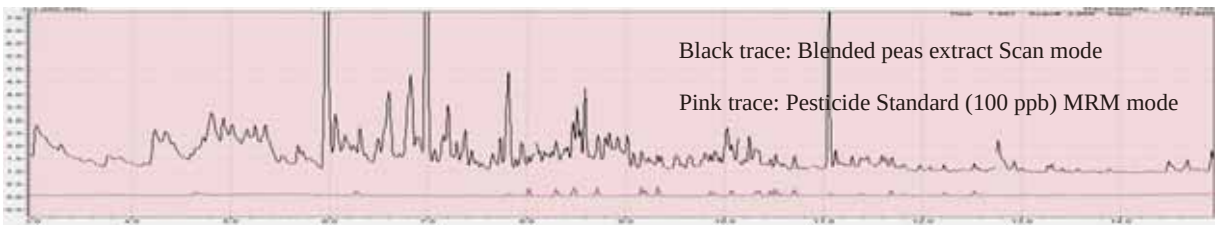


Fig. 4.1.1 Total Ion Chromatograms of Blended Peas Extract and Pesticides Standard (100 ppb)

4.1 Analysis of Organophosphorus Pesticides in Baby Foods (2) - GC/MS/MS

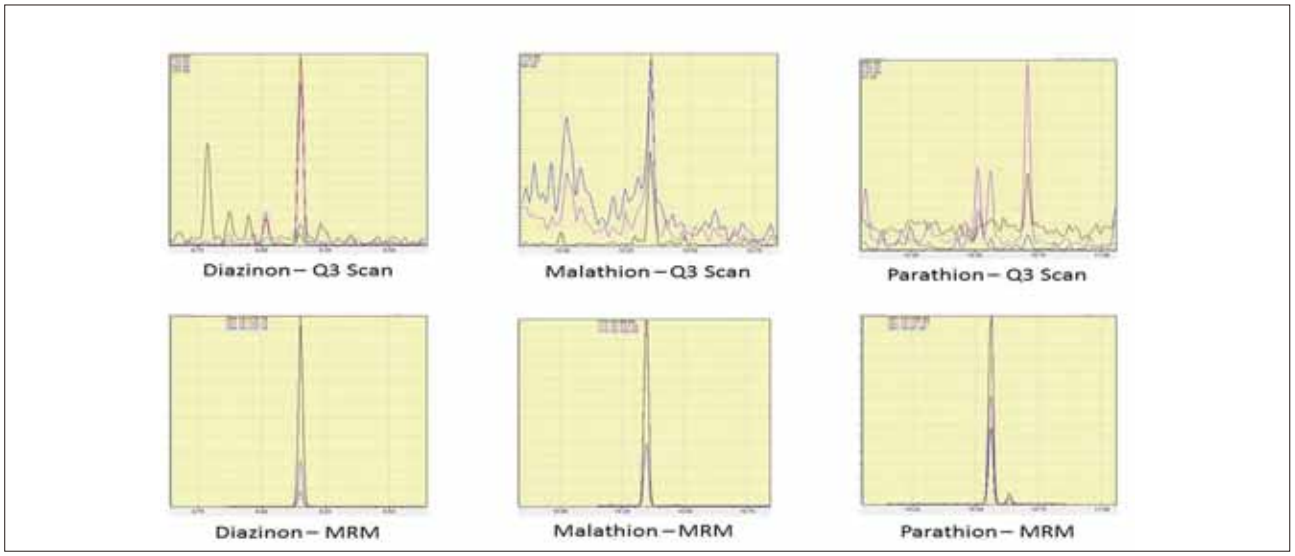


Fig. 4.1.2 Extracted Ion and MRM Chromatograms for Selected Pesticides

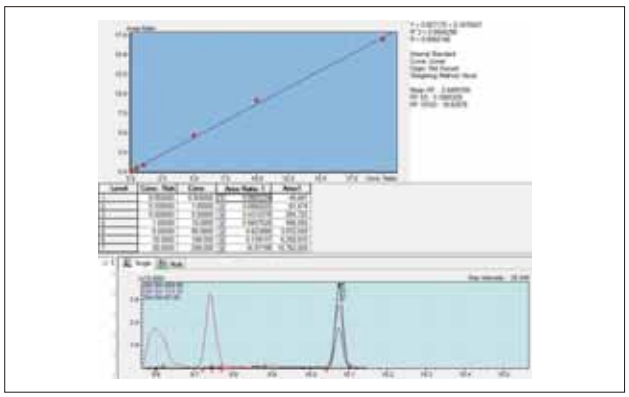


Fig. 4.1.3A Ronnel Calibration

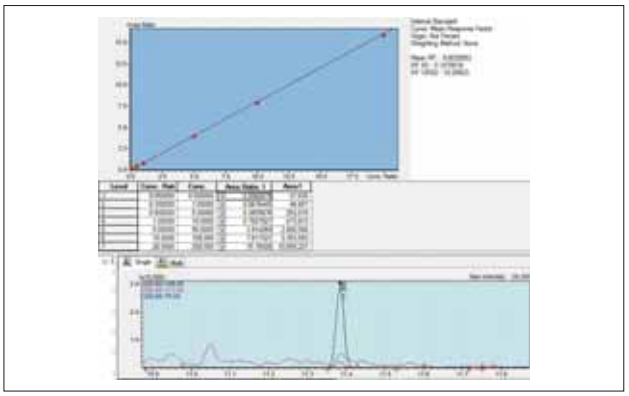


Fig. 4.1.3B Stirophos Calibration

Eight replicate injections of the 1 ng/mL and 10 ng/mL standards were analyzed to assess the precision of the analytical method and the accuracy of measurement near the low end of the calibration range. The mean measured concentrations and % RSD for the 1 ng/mL replicate analyses were 0.75-1.25 ng/mL and < 20 %; mean concentrations and % RSD for the 10 ng/mL replicate analyses were 8.0-11.0 ng/mL and < 10 %, respectively. [2)]

■Conclusion

Detection of the organophosphorus pesticides was demonstrated at low ng/mL (ppb) levels in baby food extract; linear calibration was demonstrated from 0.5-200 ng/mL. Precision and accuracy were demonstrated by replicate analyses of matrix spiked aliquots at 1 and 10 ng/mL. Calibration was conducted in the blended peas QuEChERS extract, and provided accurate, repeatable results for the sample matrix. A Shimadzu GCMS-TQ8030 system operated in the MRM mode was shown to be a rapid, sensitive, and selective technique for analysis of organophosphorus pesticides in baby foods.

[References]

1) AOAC Official Method 2007.01, Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate (2007)
2) Shimadzu Application News GCMS-1304

4.2 Analysis of Pesticides in Baby Foods (1) - GC/MS/MS

Introduction

Contamination of food products with pesticides is a growing global concern, particularly for baby foods. GC/MS/MS operated in the Multiple Reaction Monitoring (MRM) mode is a technique of choice for analysis of trace contaminants in complex matrices because of its improved sensitivity and selectivity compared to single quadrupole GCMS.

Experimental

Samples were prepared using the QuEChERS (Quick Easy Cheap Effective Rugged and Safe) sample preparation method[1]. Analyses were conducted with a Shimadzu GCMS-TQ8030 GC/MS/MS operated in the MRM mode using conditions detailed Shimadzu Application News GCMS-1402[2]. MRM transitions and optimized collision energies detailed in the Shimadzu GC/MS/MS Pesticide Database.[3] Individual 5-point calibration curves were prepared for 36 pesticides from several chemical classes using the matrix-matched internal standard procedure. A sample of organic blended pears was used to minimize contribution of analytes from the sample matrix. Selectivity of the MRM technique was evaluated as a function of mass spectral resolution on Q1 and Q3 of the GCMS-TQ8030.

Results and Discussion

Chromatography

The chromatography of pesticides and the specificity of the GCMS-TQ8030 operated in the MRM mode been explained previously.[4] A chromatogram showing the separation of the 36 pesticides is shown in Fig. 4.2.1.

Calibration and Assessment of Precision with Variable MS Resolution

One of the purposes of this study was to evaluate calibration linearity, analytical precision, and qualitative specificity in the MRM mode with variable resolution on Q1 and Q3. Variable resolution settings can be selected for the MRM transitions for a given analyte. As resolution is increased (FWHM is decreased), specificity is increased and signal intensity is decreased. Resolution on Q1 and Q3 has minimal effect on electronic noise, but has considerable effect on chromatographic interference, which can be considered chemical noise. Depending on the interference for a specific analyte in a given sample matrix, optimum sensitivity (signal/noise) relative to resolution is empirical.

Calibration was conducted at three resolution settings on Q1 and Q3 using the matrix-matched internal standard procedure. Five calibration standards were prepared in a blended pears extract over the range of 1-200 ng/mL (ppb). Response factors were calculated and relative standard deviation (RSD) determined by the GCMSsolution software. The precision of the calibration was evaluated using the RSD of the response factors and the correlation coefficient (r) for each of the analytes. In general, the % RSD was < 25 % and correlation coefficient values for the multi-point calibration were > 0.999 when using the resolution setting Q1 – Unit; Q3 – Unit.

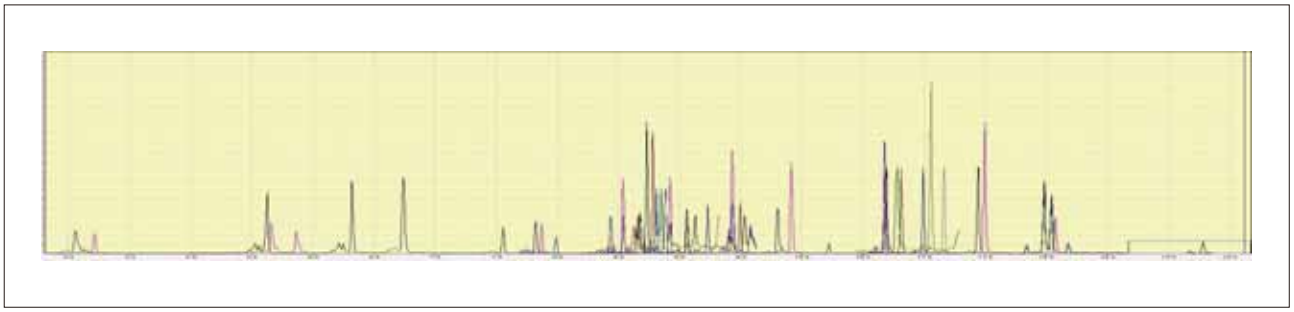


Fig. 4.2.1 Total Ion Chromatogram of Pesticide Standard – MRM Mode

4.2 Analysis of Pesticides in Baby Foods (2) - GC/MS/MS

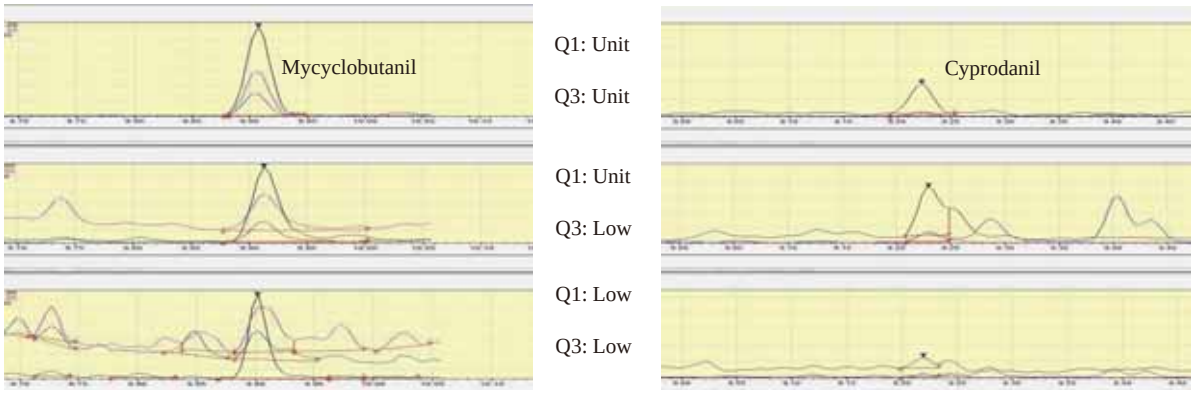


Fig. 4.2.2 MRM Chromatograms for Trace Pesticides at Three Settings of MS Resolution

After acquisition of each of the multi-point calibrations, eight replicate injections each of the 1.0 and 5.0 ng/mL standards were analyzed to assess the precision and accuracy of measurement near the low end of the calibration range. The mean concentration and RSD for the replicate analyses are generally less than 20 % at 1 ppb and less than 10 % at 5 ppb.[2] The precision of the measurements was considered acceptable for this application.

Chromatographic interferences show considerable variability between compounds, but are minimized using Unit/Unit resolution setting (Q1 – unit; Q3 – unit). For some compounds, striking improvement in selectivity is obtained with increasing resolution; two examples are shown in Fig. 4.2.2.

■Conclusion

Detection of pesticides was demonstrated at low ng/mL (ppb) levels in a QuEChERS extract of blended pears, and linear, matrix-matched calibration was demonstrated from 1-200 ng/mL. Operation of the GCMS-TQ8030 in the MRM mode provided accurate, precise results for the sample matrix. Precision and accuracy were demonstrated by replicate analyses of matrix spiked aliquots at 1 and 5 ng/mL.

The results demonstrate that chromatographic interferences can be significantly reduced by increasing the mass spectral resolution. Optimum sensitivity (signal/noise) relative to resolution is empirical and variable with each analyte and matrix.

[References]

1) AOAC Official Method 2007.01, Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate (2007)
2) Analysis of Pesticides in Baby Foods Using a GCMS-TQ8030 GC/MS/MS, Part Ⅱ Shimadzu Application News No. GCMS-1402 (December, 2013)
3) Shimadzu GCMSMS Pesticide Database (October, 2012)
4) Analysis of Organophosphorus Pesticides in Baby Foods Using a Triple-Quadrupole GC/MS/MS System Shimadzu Application News No. GCMS-1304 (February, 2013)

4.3 Analysis of Pesticides in Citrus Oil (1) - GC/MS/MS

Introduction

Contamination of consumer products with pesticides is a growing concern. Triple quadrupole GC/MS/MS operated in the Multiple Reaction Monitoring (MRM) mode has emerged as a technique of choice for analysis of trace level contaminants in complex matrices such as orange oil. A Shimadzu GCMS-TQ8030 was used for trace analysis of 47 pesticides of various chemical classes using the instrument configuration and operating parameters described previously.[1] Results were evaluated for calibration linearity, analytical precision, sensitivity, and specificity.

Experimental

Samples were diluted 10-fold in dichloromethane for analysis. The analyses were conducted using a Shimadzu GCMS-TQ8030 triple quadrupole GC/MS/MS operated in the multiple reaction monitoring (MRM) mode. Calibration was conducted using the matrix-matched internal standard procedure. Details of the analytical conditions are summarized in Shimadzu Application News.[2] MRM transitions details were taken from the Shimadzu pesticide database.[3]

A sample of organic orange oil was used as the sample matrix so it would be free from background pesticide contamination.

Results and Discussion

Chromatography

The total ion chromatogram (TIC) acquired in the Q3 full-scan mode for the pesticide standard is shown in Fig. 4.3.1A, and a chromatogram for organic orange oil is shown in Fig. 4.3.1B, and shows a broad, intense signal for limonene before 7.5 min. and intense chromatographic peaks from 7.5 - 12 min.

Assessment of Optimum Mass Spectral Resolution

Variable resolution settings can be selected for the MRM transitions for a given analyte. As MS resolution is increased (FWHM is decreased), specificity is increased and signal intensity is decreased. Resolution on Q1 and Q3 has minimal effect on electronic noise, but has considerable effect on chromatographic interference, which can be considered "chemical noise". Depending on the interference for a specific analyte in a given sample matrix, optimum sensitivity (signal/noise) relative to resolution is empirical. The chromatograms of all of the analytes were examined visually to assess optimum resolution. Based on visual comparison of the results, the resolution setting of Q1: unit; Q3: unit was chosen for the analyses.

Qualitative Specificity and Chromatographic Interferences

MRM chromatograms for the analytes were displayed for calibration standards at the lowest calibration level. For many of the analytes, minimal chromatographic interference was observed at the lowest level, which was defined as the limit of quantitation (LOQ). For some other analytes, however, significant interference is observed even at higher concentrations, despite the specificity of the MRM mode, owing to the complexity of the orange oil matrix. Examples of interferences are presented Figures 4.3.2A and 4.3.2B.

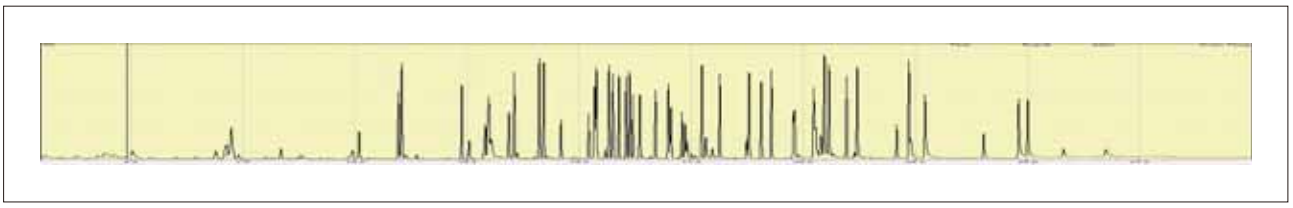


Fig. 4.3.1A Total Ion Chromatogram of Pesticide Standard – Q3 Scan Mode

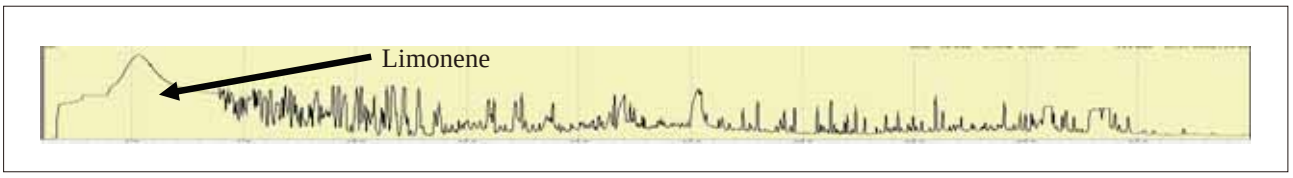


Fig. 4.3.1B Total Ion Chromatogram of Organic Orange Oil – Q3 Scan Mode

4.3 Analysis of Pesticides in Citrus Oil (2) - GC/MS/MS

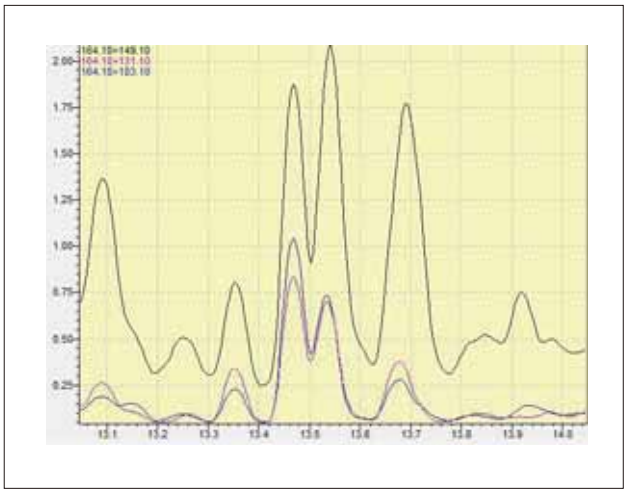


Fig. 4.3.2A MRM Chromatogram Carbofuran - 50 ppb in Orange Oil

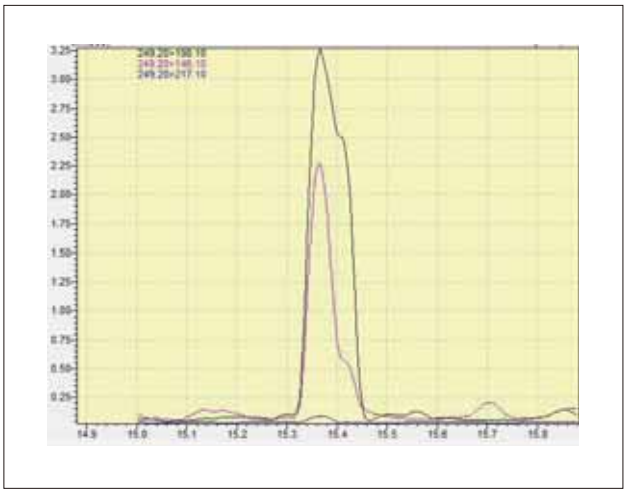


Fig. 4.3.2B MRM Chromatogram Metalaxyl - 50 ppb in Orange Oil

Calibration and Assessment of Precision

Calibration was conducted using the matrix-matched internal standard procedure. Nine calibration standards were prepared in diluted organic orange oil over the range of 0.5-500 pg for each analyte (corresponding to 5-5000 ppm in the orange oil samples. The precision of the calibration was evaluated using the RSD of the response factors (< 20 %) and the correlation coefficient (r) (> 0.999) for each of the analytes. After the multi-point calibration, ten replicate injections of each of the lowest level standards were analyzed to assess the precision of measurement (< 10 %) and accuracy (80-120 % for most of the analytes) near the low end of the calibration range. In a few cases, chromatographic interferences contributed to high-biased results (> 120 %). The precision and accuracy for the multi-point calibration and the replicate analyses were considered acceptable for this application.

■Conclusion

Detection of pesticides was demonstrated at low ng/mL (ppb) levels in orange oil using a Shimadzu GCMS-TQ8030. Calibration was conducted using the matrix-matched internal standard procedure. Nine calibration standards were prepared and analyzed in diluted organic orange oil over the range of 0.5-500 pg. Precision and accuracy were demonstrated by replicate analyses of matrix spiked aliquots at 5, 10 and 50 ng/mL. Results were evaluated for calibration linearity, analytical precision, sensitivity, and specificity. A Shimadzu GCMS-TQ8030 system was shown to be a rapid, sensitive, and selective technique for analysis of various classes of pesticides in orange oil. Operation of the GC/MS/MS in the MRM mode provided accurate, precise results for an extremely complex sample matrix.

[References]

1) Shimadzu Application News No. GCMS-1304 (February, 2013)
2) Shimadzu Application News No. GCMS-1404 (February, 2014)
3) Shimadzu GCMSMS Pesticide Database (October, 2012)

4.4 Simultaneous Analysis of Residual Pesticides in Foods via the QuEChERS Method (1) - GC/MS/MS

■Explanation

Analytical standards (0.001 mg/L to 0.1 mg/L), as well as samples (0.01 mg/L) created by pretreating paprika with the QuEChERS method and then adding pesticides to the resulting solution, were measured using GC-MS/MS.

■Experimental

The European Union Reference Laboratory (EURL) has reported their results on evaluating the validity of residual pesticide analysis utilizing GC-MS/MS and LC-MS/MS[1]. In their report, the measurement of 66 pesiticides using GC-MS/MS was recommended. Here we present selected results of analysis of these pesticides using the triple quadrupole GCMS-TQ8030.

■Analytical Conditions

Instrument : GCMS-TQ8030
Column : Rxi-5Sil MS (30 m length, 0.25 mm I.D., df = 0.25 µm)
GlassLiner : Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek Corporation, catalog # 567366)

[GC]
Injection Temp. : 250 °C
Column Temp. : 70 °C (2 min) - (25 °C/min) - 150 °C - (3 °C/min) - 200 °C - (8 °C/min) - 280 °C/min (10 min)
Injection Mode : Splitless
Flow Control Mode : Linear velocity (58.1 cm/sec.)
Injection Volume : 1 µL

[MS]
Interface Temp. : 250 °C
Ion Source Temp. : 230 °C
Data Acquisition Mode : MRM (See the below.)

MRM Monitoring *m/z*

	Quantitative Transition		Qualitative Transition					Quantitative Transition		Qualitative Transition			
Compound Name	Precursor>Product	CE (V)	Precursor>Product	CE (V)			Compound Name	Precursor>Product	CE (V)	Precursor>Product	CE (V)		
Diphenylamine	169.10>77.00	26	169.10>115.10	30			Buprofezin	172.10>57.10	18	105.10>104.10	4		
Ethoprophos	200.00>157.90	6	200.00>114.00	14	200.00>97.00	26	Bupirimate	273.10>193.20	8	273.10>108.00	18		
Chlorpropham	213.10>171.10	6	213.10>127.10	18			beta-Endosulfan	240.90>205.90	14	238.90>203.90	14		
Trifluralin	306.10>264.00	8	264.10>206.10	8	264.10>160.10	18	Oxadixyl	163.10>132.10	10	163.10>117.10	24		
Dicloran	206.00>176.00	12	206.00>124.00	26	176.00>148.00	12	Ethion	231.00>174.90	14	231.00>128.90	26		
Propyzamide	172.90>144.90	16	172.90>109.00	26			Triazophos	161.10>134.10	8	161.10>106.10	14		
Chlorothalonil	265.90>230.90	14	265.90>167.90	24	263.90>167.90	24	Endosulfan sulfate	386.90>252.90	10	386.90>216.90	26		
Diazinon	304.10>179.10	12	179.20>137.20	18			Propiconazole-1	259.10>190.90	8	259.10>172.90	18	259.10>69.10	12
Pyrimethanil	199.10>184.10	14	199.10>158.10	14			Propiconazole-2	259.10>190.90	8	259.10>172.90	18	259.10>69.10	12
Tefluthrin	197.10>141.10	26	177.10>127.10	32			Tebuconazole	252.10>127.00	24	250.10>125.10	24		
Pirimicarb	238.20>166.10	10	166.10>96.00	14			Iprodione	314.10>244.90	12	314.10>56.10	24		
Chlorpyrifos-methyl	285.90>270.90	12	285.90>93.00	22			Bromopropylate	340.90>184.90	18	182.90>154.90	16		
Vinclozolin	212.10>172.00	14	212.10>144.90	26	212.10>109.00	30	Bifenthrin	181.10>166.10	16	181.10>165.10	22	181.10>153.10	10
Parathion-methyl	263.10>109.00	18	263.10>81.00	26			Fenpropathrin	265.10>210.10	12	181.10>152.10	24	181.10>127.10	26
Tolclofos-methyl	265.00>249.90	12	265.00>93.00	24			Fenazaquin	160.20>145.10	8	145.20>115.10	24	145.20>91.10	24
Metalaxyl	206.20>162.10	8	206.20>132.10	18			Tebufenpyrad	333.20>276.10	8	333.20>171.00	22		
Fenitrothion	277.10>125.00	18	277.10>109.00	18			Tetradifon	355.90>158.90	12	353.90>159.00	12	228.90>200.90	14
Pirimiphos-methyl	305.10>290.10	12	290.10>125.00	24			Phosalone	182.00>138.00	8	182.00>111.00	18	182.00>102.10	18
Dichlofluanid	332.00>167.10	6	224.00>123.00	12			Pyriproxyfen	136.10>96.00	12	136.10>78.00	24		
Malathion	173.10>117.00	12	173.10>99.00	18			Cyhalothrin	181.10>152.10	24	163.10>127.00	14	163.10>91.00	22
Chlorpyrifos	196.90>168.90	14	196.90>107.00	26			Fenarimol	251.00>139.00	18	139.10>111.00	16		
Fenthion	278.10>125.00	22	278.10>109.00	18			Acrinathrin	289.10>93.10	12	181.10>152.10	24	208.10>181.10	8
Parathion	291.10>109.00	14	291.10>81.00	26			Permethrin-1	183.10>168.10	12	183.10>153.10	18	183.10>115.10	24
Tetraconazole	336.10>218.00	18	336.10>204.00	26			Pyridaben	147.20>132.10	14	147.20>117.10	22		
Pendimethalin	252.20>162.10	12	252.20>161.10	12			Permethrin-2	183.10>168.10	12	183.10>153.10	18	183.10>115.10	24
Cyprodinil	225.20>224.10	6	224.20>208.10	18			Cyfluthrin-1	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
(E)-Chlorfenvinphos	323.10>266.90	14	267.00>159.00	18			Cyfluthrin-2	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Tolylfluanid	137.10>91.00	18	137.10>65.00	26			Cyfluthrin-3	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Fipronil	367.00>227.90	26	367.00>212.90	26			Cyfluthrin-4	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Captan	79.00>77.00	8	79.00>51.00	22			Cypermethrin-1	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
(Z)-Chlorfenvinphos	323.10>266.90	14	267.00>159.00	18			Cypermethrin-2	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Phenthoate	274.10>125.00	18	274.10>121.10	12			Cypermethrin-3	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Folpet	147.10>103.10	10	147.10>76.00	26			Cypermethrin-4	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Procymidone	283.10>96.10	12	283.10>67.10	24			Ethofenprox	163.20>135.00	10	163.20>107.10	18		
Methidathion	145.10>85.00	8	145.10>58.00	18			Fenvalerate-1	125.10>99.00	22	125.10>89.00	22		
alpha-Endosulfan	240.90>205.90	14	238.90>203.90	16			tau-Fluvarlinate-1	250.10>200.10	16	250.10>55.00	18		
Mepanipyrim	222.20>220.10	8	222.20>193.10	26			Fenvalerate-2	125.10>99.00	22	125.10>89.00	22		
Profenofos	337.10>266.80	16	207.90>63.00	26			tau-Fluvarlinate-2	250.10>200.10	16	250.10>55.00	18		
Myclobutanil	179.10>152.00	8	179.10>125.00	16			Deltamethrin-1	252.90>93.10	18	181.10>152.10	24		
Flusilazole	233.10>165.10	18	233.10>152.10	18			Deltamethrin-2	252.90>93.10	18	181.10>152.10	24		

4.4 Simultaneous Analysis of Residual Pesticides in Foods via the QuEChERS Method (2) - GC/MS/MS

■Results

Calibration curves for each pesticide obtained by analyzing six calibration standards (0.001 mg/L to 0.1 mg/L), the mass chromatograms for the 0.01 mg/L samples, and the area repeatability (n=6) for each pesticide obtained from the pesticide-spiked samples (0.01 mg/L) are shown below.

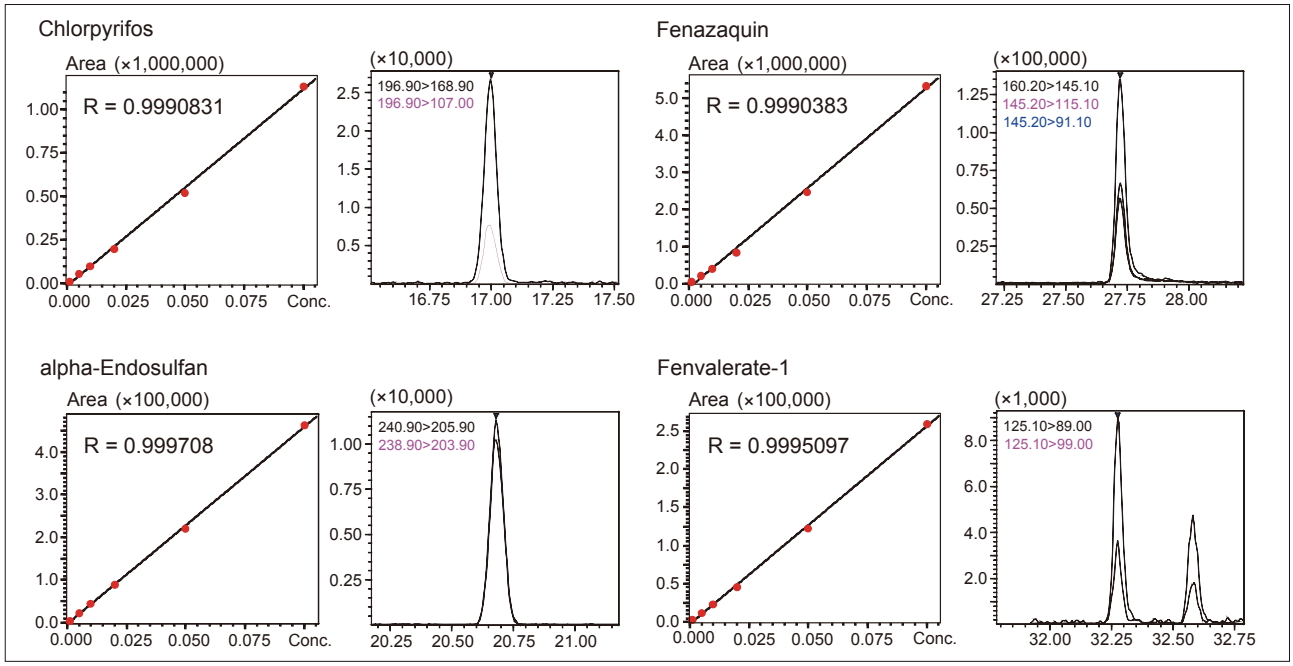


Fig. 4.4.1 Calibration Curves for Each Pesticide and the Mass Chromatograms for the 0.01 mg/L Samples

Table 4.4.1 Area Reproducibility for Each Pesticide (n=6)

Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD
Diphenylamine	4.99	Chlorpyrifos	5.23	Buprofezin	4.92	Fenarimol	5.16
Ethoprophos	4.95	Fenthion	5.75	Bupirimate	5.47	Acrinathrin	2.03
Chlorpropham	6.26	Parathion	6.93	beta-Endosulfan	6.29	Permethrin-1	6.34
Trifluralin	5.33	Tetraconazole	6.96	Oxadixyl	5.74	Pyridaben	7.11
Dicloran	6.49	Pendimethalin	6.29	Ethion	6.18	Permethrin-2	6.24
Propyzamide	5.52	Cyprodinil	5.21	Triazophos	3.45	Cyfluthrin-1	4.44
Chlorothalonil	4.46	(E)-Chlorfenvinphos	5.35	Endosulfan sulfate	4.26	Cyfluthrin-2	3.77
Diazinon	5.45	Tolylfluanid	4.81	Propiconazole-1	6.02	Cyfluthrin-3	7.35
Pyrimethanil	3.18	Fipronil	6.76	Propiconazole-2	5.56	Cyfluthrin-4	8.19
Tefluthrin	5.13	Captan	5.74	Tebuconazole	7.59	Cypermethrin-1	8.58
Pirimicarb	5.00	(Z)-Chlorfenvinphos	5.52	Iprodione	1.72	Cypermethrin-2	3.71
Chlorpyrifos-methyl	5.27	Phenthoate	6.40	Bromopropylate	5.71	Cypermethrin-3	8.08
Vinclozolin	6.33	Folpet	6.56	Bifenthrin	5.29	Cypermethrin-4	2.48
Parathion-methyl	5.81	Procymidone	6.40	Fenpropathrin	4.00	Ethofenprox	5.03
Tolclofos-methyl	4.89	Methidathion	6.17	Fenazaquin	4.84	Fenvalerate-1	4.20
Metalaxyl	5.43	alpha-Endosulfan	6.27	Tebufenpyrad	5.62	tau-Fluvarlinate-1	2.16
Fenitrothion	5.10	Mepanipyrim	6.41	Tetradifon	6.09	Fenvalerate-2	5.65
Pirimiphos-methyl	5.35	Profenofos	5.92	Phosalone	5.90	tau-Fluvarlinate-2	2.14
Dichlofluanid	4.04	Myclobutanil	5.46	Pyriproxyfen	5.16	Deltamethrin-1	7.58
Malathion	6.31	Flusilazole	5.63	Cyhalothrin	5.38	Deltamethrin-2	7.32

[Reference]

1) EURL-FV Multiresidue Method using QuEChERS followed by GC-QqQ/MS/MS and LC-QqQ/MS/MS for Fruits and Vegetables (European Reference Laboratory, 2010-M1)

4.5 Scan/MRM Analysis of Residual Pesticides in Foods (1) - GC/MS/MS

■Explanation

The GCMS-TQ8030 is a triple quadrupole GC-MS/MS system equipped with scan/MRM mode to allow simultaneous scan and MRM data measurements. Here we introduce the results of an investigation using the scan/MRM mode, where target pesticides were quantitatively determined using the MRM data, and concentrations of the untargeted pesticides were estimated by applying the scan data to the Compound Composer Database Software Ver. 2.

■Experimental

For the evaluation, analytical standards (0.001 mg/L to 0.1 mg/L) were used, as well as samples (0.01 mg/L) created by pretreating paprika with the QuEChERS method, and then adding pesticides to the obtained solution. The pesticides specified as targets and their transitions were those recommended in the results of the validity evaluations by the European Reference Laboratory[1)].

■Analytical Conditions

Instrument : GCMS-TQ8030
Column : Rxi-5Sil MS (30 m length, 0.25 mm I.D., df = 0.25 µm)
Glass Liner : Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek Corporation, catalog # 567366)

[GC]
Injection Temp. : 250 °C
Column Temp. : 40 °C (2 min) - (8 °C/min) - 310 °C (5 min)
Injection Mode : Splitless
Flow Control Mode : Linear velocity (40.0 cm/sec)
Injection Volume : 1 µL

[MS]
Interface Temp. : 300 °C
Ion Source Temp. : 200 °C
Data Acquisition Mode : Scan/MRM
Event Time : 0.1 sec (Scan), 0.3 sec (MRM)
Scan Mass Range : *m/z* 50 – 500
Scan Speed : 5,000 u/sec

MRM Monitoring *m/z*

Compound Name	Quantitative Transition Precursor>Product	CE (V)	Qualitative Transition Precursor>Product	CE (V)		
Diphenylamine	169.10>77.00	26	169.10>115.10	30		
Ethoprophos	200.00>157.90	6	200.00>114.00	14	200.00>97.00	26
Chlorpropham	213.10>171.10	6	213.10>127.10	18		
Trifluralin	306.10>264.00	8	264.10>206.10	8	264.10>160.10	18
Dicloran	206.00>176.00	12	206.00>124.00	26	176.00>148.00	12
Propyzamide	172.90>144.90	16	172.90>109.00	26		
Chlorothalonil	265.90>230.90	14	265.90>167.90	24	263.90>167.90	24
Diazinon	304.10>179.10	12	179.20>137.20	18		
Pyrimethanil	199.10>184.10	14	199.10>158.10	14		
Tefluthrin	197.10>141.10	26	177.10>127.10	32		
Pirimicarb	238.20>166.10	10	166.10>96.00	14		
Chlorpyrifos-methyl	285.90>270.90	12	285.90>93.00	22		
Vinclozolin	212.10>172.00	14	212.10>144.90	26	212.10>109.00	30
Parathion-methyl	263.10>109.00	18	263.10>81.00	26		
Tolclofos-methyl	265.00>249.90	12	265.00>93.00	24		
Metalaxyl	206.20>162.10	8	206.20>132.10	18		
Fenitrothion	277.10>125.00	18	277.10>109.00	18		
Pirimiphos-methyl	305.10>290.10	12	290.10>125.00	24		
Dichlofluanid	332.00>167.10	6	224.00>123.00	12		
Malathion	173.10>117.00	12	173.10>99.00	18		
Chlorpyrifos	196.90>168.90	14	196.90>107.00	26		
Fenthion	278.10>125.00	22	278.10>109.00	18		
Parathion	291.10>109.00	14	291.10>81.00	26		
Tetraconazole	336.10>218.00	18	336.10>204.00	26		
Pendimethalin	252.20>162.10	12	252.20>161.10	12		
Cyprodinil	225.20>224.10	6	224.20>208.10	18		
(E)-Chlorfenvinphos	323.10>266.90	14	267.00>159.00	18		
Tolylfluanid	137.10>91.00	18	137.10>65.00	26		
Fipronil	367.00>227.90	26	367.00>212.90	26		
Captan	79.00>77.00	8	79.00>51.00	22		
(Z)-Chlorfenvinphos	323.10>266.90	14	267.00>159.00	18		
Phenthoate	274.10>125.00	18	274.10>121.10	12		
Folpet	147.10>103.10	10	147.10>76.00	26		
Procymidone	283.10>96.10	12	283.10>67.10	24		
Methidathion	145.10>85.00	8	145.10>58.00	18		
alpha-Endosulfan	240.90>205.90	14	238.90>203.90	16		
Mepanipyrim	222.20>220.10	8	222.20>193.10	26		
Profenofos	337.10>266.80	16	207.90>63.00	26		
Myclobutanil	179.10>152.00	8	179.10>125.00	16		
Flusilazole	233.10>165.10	18	233.10>152.10	18		

Compound Name	Quantitative Transition Precursor>Product	CE (V)	Qualitative Transition Precursor>Product	CE (V)		
Buprofezin	172.10>57.10	18	105.10>104.10	4		
Bupirimate	273.10>193.20	8	273.10>108.00	18		
beta-Endosulfan	240.90>205.90	14	238.90>203.90	14		
Oxadixyl	163.10>132.10	10	163.10>117.10	24		
Ethion	231.00>174.90	14	231.00>128.90	26		
Triazophos	161.10>134.10	8	161.10>106.10	14		
Endosulfan sulfate	386.90>252.90	10	386.90>216.90	26		
Propiconazole-1	259.10>190.90	8	259.10>172.90	18	259.10>69.10	12
Propiconazole-2	259.10>190.90	8	259.10>172.90	18	259.10>69.10	12
Tebuconazole	252.10>127.00	24	250.10>125.10	24		
Iprodione	314.10>244.90	12	314.10>56.10	24		
Bromopropylate	340.90>184.90	18	182.90>154.90	16		
Bifenthrin	181.10>166.10	16	181.10>165.10	22	181.10>153.10	10
Fenpropathrin	265.10>210.10	12	181.10>152.10	24	181.10>127.10	26
Fenazaquin	160.20>145.10	8	145.20>115.10	24	145.20>91.10	24
Tebufenpyrad	333.20>276.10	8	333.20>171.00	22		
Tetradifon	355.90>158.90	12	353.90>159.00	12	228.90>200.90	14
Phosalone	182.00>138.00	8	182.00>111.00	18	182.00>102.10	18
Pyriproxyfen	136.10>96.00	12	136.10>78.00	24		
Cyhalothrin	181.10>152.10	24	163.10>127.00	14	163.10>91.00	22
Fenarimol	251.00>139.00	18	139.10>111.00	16		
Acrinathrin	289.10>93.10	12	181.10>152.10	24	208.10>181.10	8
Permethrin-1	183.10>168.10	12	183.10>153.10	18	183.10>115.10	24
Pyridaben	147.20>132.10	14	147.20>117.10	22		
Permethrin-2	183.10>168.10	12	183.10>153.10	18	183.10>115.10	24
Cyfluthrin-1	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Cyfluthrin-2	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Cyfluthrin-3	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Cyfluthrin-4	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Cypermethrin-1	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Cypermethrin-2	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Cypermethrin-3	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Cypermethrin-4	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Ethofenprox	163.20>135.00	10	163.20>107.10	18		
Fenvalerate-1	125.10>99.00	22	125.10>89.00	22		
tau-Fluvarlinate-1	250.10>200.10	16	250.10>55.00	18		
Fenvalerate-2	125.10>99.00	22	125.10>89.00	22		
tau-Fluvarlinate-2	250.10>200.10	16	250.10>55.00	18		
Deltamethrin-1	252.90>93.10	18	181.10>152.10	24		
Deltamethrin-2	252.90>93.10	18	181.10>152.10	24		

4.5 Scan/MRM Analysis of Residual Pesticides in Foods (2) - GC/MS/MS

■Results

An example of the results of the analysis of the analytical standards (0.001 mg/L to 0.1 mg/L) and the pesticide-spiked samples (0.01 mg/L) in scan/MRM mode are shown in Fig. 4.5.1. As with Procymidone, shown in Fig. 4.5.1, strict quantification of the targeted pesticides could be performed by creating calibration curves from the MRM data. Furthermore, since the scan data was sampled simultaneously, the pesticides could be confirmed from the mass spectrum.

For the untargeted pesticides, data analysis was performed utilizing the Compound Composer Database Software (P/N: 225-13106-92). The simultaneous analysis database software contains information (mass spectra, retention times, and calibration curves) on more than 450 pesticides. As a result, it is possible to identify pesticides from their estimated retention times and mass spectra without using analytical standards, and then calculate semi-quantitative values from the calibration curves. Also in this investigation, it was possible to confirm the detection and semi-quantification of untargeted pesticides such as Quinoxyfen.

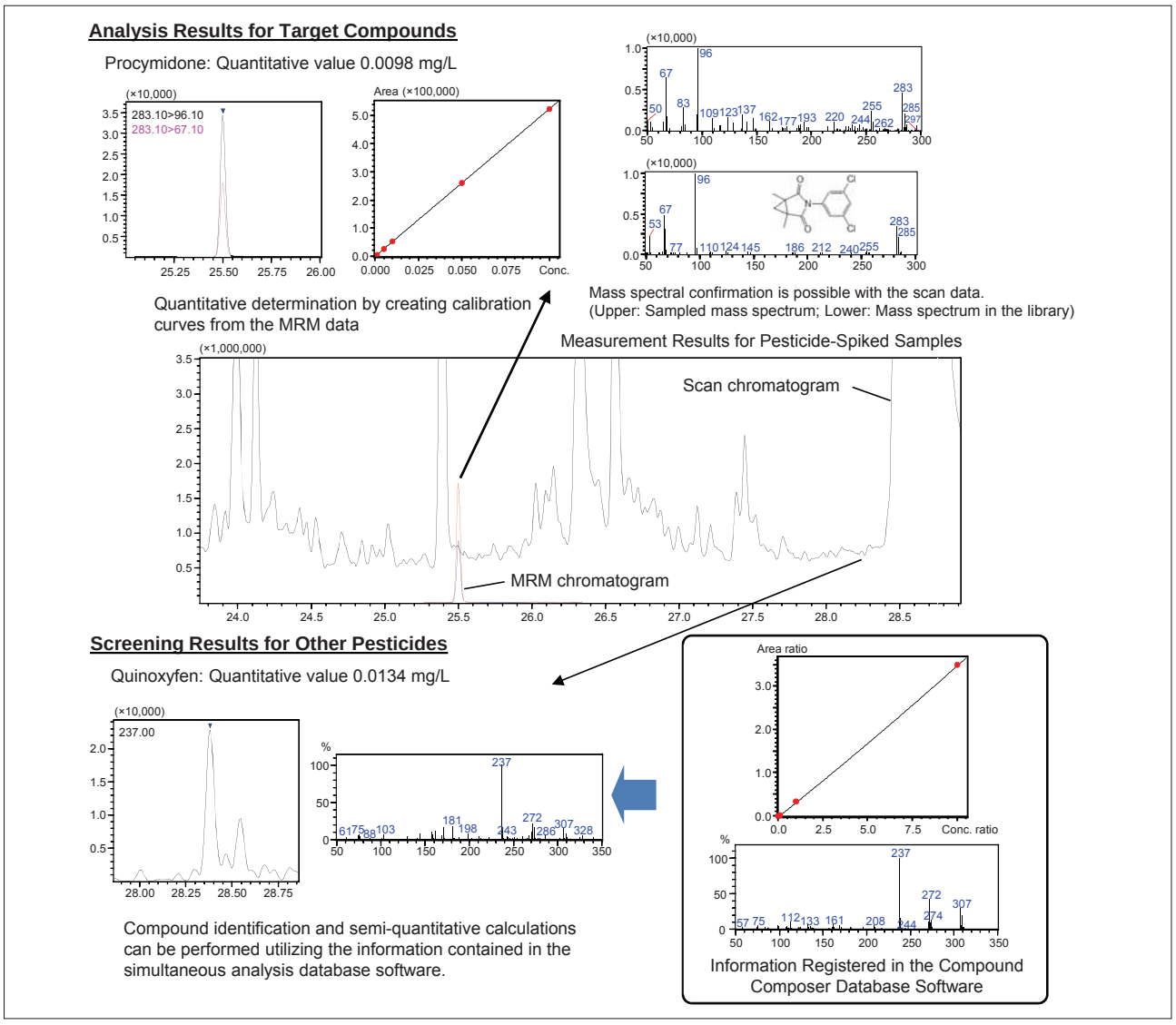


Fig. 4.5.1 Scan/MRM Analysis Results

[Reference]

1) EURL-FV Multiresidue Method using QuEChERS followed by GC-QqQ/MS/MS and LC-QqQ/MS/MS for Fruits and Vegetables (European Reference Laboratory, 2010-M1)

4.6 Screening of Residual Pesticides in Food with Two Different Columns (1) - GC/MS

■Explanation

In recent years, with increases in the number of pesticides and the diversification of substances under investigation, there have been calls for quick and high-accuracy screening for residual pesticides in foods using GC/MS. The Quick-DB database includes mass spectra, retention times, and calibration curves for 478 pesticide components. It can be used to quickly calculate semi-quantitative values without requiring analytical standards. If a pesticide peak is detected, it is essential to check for potential interference from co-eluting contaminants to insure highly accurate screening. One of the ways for checking is to analyze the samples with two columns with different types of stationary phase. For this purpose, the Twin Line MS system is very useful because it enables the installation of two types of columns to one MS. The Quick-DB contains information compatible with two different columns, so that it can also be applied to the Twin Line MS system. Here, residual pesticides in foods were analyzed by applying Quick-DB and the Twin Line MS system.

■Experimental

Commercially-available oranges and soya beans were processed with the QuEChERS method using Restek Q-sep™. A mixture of 138 pesticides was added to the sample solutions, with the concentrations adjusted to 10 ng/mL. The pesticide-spiked samples were subjected to Scan/SIM analysis using the analysis conditions stored in Quick-DB. Frequently detected components were analyzed with high sensitivity in SIM mode. For components with low detection frequency, a comprehensive analysis was performed in Scan mode.

■Analytical Conditions

Instruments	: GCMS-QP2010 Ultra with Twin Line MS System
Column 1	: Rxi-5Sil MS (30 mL., 0.25 mm I.D., df = 0.25 µm) (Restek Corporation, P/N: 13623)
Column 2	: Rtx-200MS (30 m L., 0.25 mm I.D., df = 0.25 µm) (Restek Corporation, P/N: 15623)
Glass Insert	: Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek Corporation, P/N: 567366)

[GC]

Injection Temp.	: 250 °C
Column Temp.	: 60 °C (1 min) - (25 °C/min) - 160 °C - (4 °C/min) - 240 °C - (10 °C/min) - 290 °C (11 min)
Injection Mode	: Splitless
High-Pressure Injection	: 250 kPa (1.5 min)
Carrier Gas Control	: Linear velocity (40.0 cm/sec)
Injection Volume	: 2 µL

[MS]

Interface Temp.	: 300 °C
Ion Source Temp.	: 200 °C
Solvent Elution Time	: 1.5 min
Measurement Mode	: FAAST (Scan/SIM simultaneous measurement)
Scan Mass Range	: *m/z* 50 to 600
Scan Event Time	: 0.15 sec
Scan Speed	: 5,000 u/sec
SIM Event Time	: 0.3 sec

<Twin Line MS System>

The inlets of the two different columns are connect to two different injection ports and the outlets are introduced directly to the mass spectrometer interface at the same time. One column is chosen for analysis while the other, non-used column, has a reduced carrier gas flowrate. This enables application data from the different columns to be acquired without venting the MS vacuum to change columns. Moreover the retention times and retention indices are the same as a single column system. A high-capacity differential vacuum system provides the same sensitivity as that obtained by a single column system.

Fig. 4.6.1 Twin Line MS System

4.6 Screening of Residual Pesticides in Food with Two Different Columns (2) - GC/MS

■Analysis Results

The pesticide-spiked samples (10 ng/mL) were analyzed using the Twin Line MS and the data were processed utilizing the Quick-DB. Figs. 4.6.2, Figs. 4.6.3, and Figs. 4.6.4 show the obtained mass chromatograms for 3 selected compounds. The left shows those run on the Rxi-5Sil MS and the right shows those on the Rtx-200 MS. Carbaryl and Aldrin peaks were impacted by close- or co-eluting contaminants on the Rxi-5Sil MS (Fig. 4.6.2 and Fig. 4.6.3 (left side)). For these two compounds, the calculated semi-quantitative values obtained from the calibration curves stored in the Quick-DB were higher than the spike amount (10.0 ng/mL) because of the interference. However, on the Rtx-200MS (on the right), the co-eluting contaminants were chromatographically separated from Carbaryl and Aldrin peaks (Fig. 4.6.2 and Fig. 4.6.3 (right side)), and the semi-quantitative values were much more closer to 10 ng/mL spike levels. Pyrimethanil were not impacted by co-eluting contaminants with either of the columns. The results obtained from two different columns enhances the reliability of screening results. These results demonstrated that the Twin Line MS allows the reliable determination of pesticides in complex samples with the potential for matrix interference, such as processed foods. This works for a semi-quantitative values using the Quick-DB.

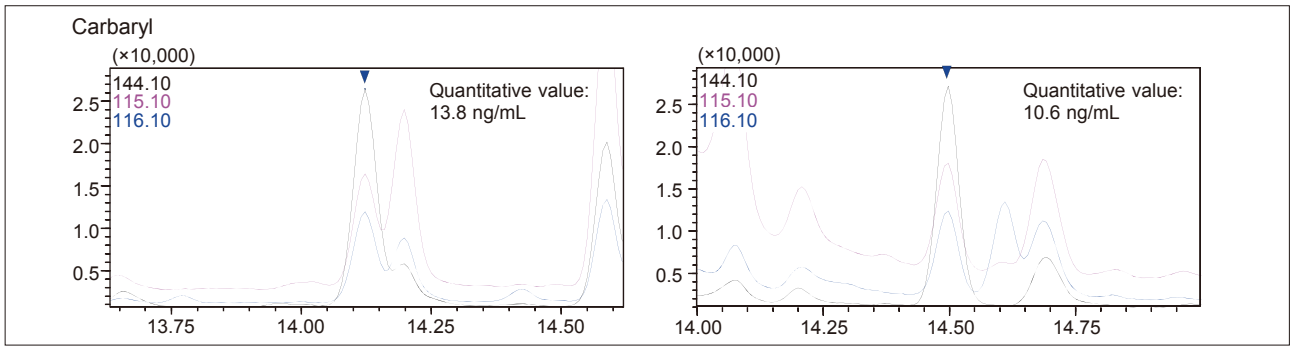


Fig. 4.6.2 Mass Chromatogram of Carbaryl (10 ng/mL) Added to Liquid Soya Beans Extract (Left: Rxi-5Sil MS; Right: Rtx-200 MS)

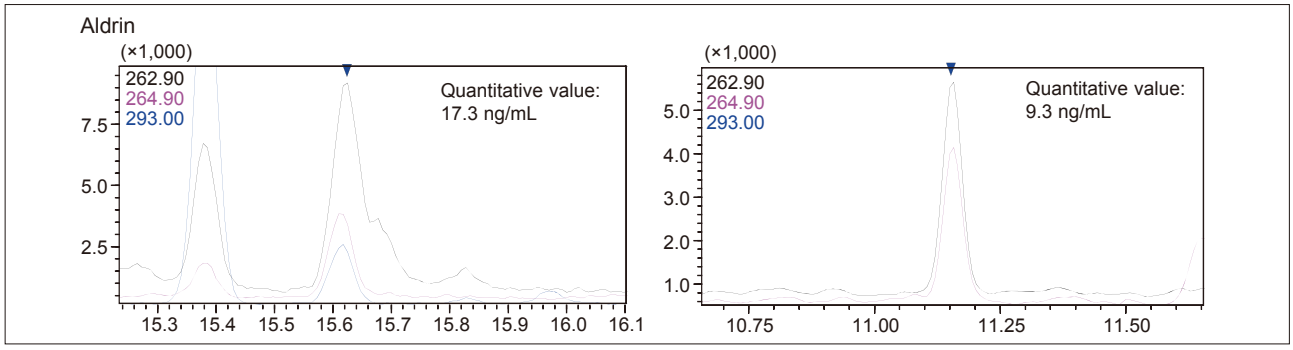


Fig. 4.6.3 Mass Chromatogram of Aldrin (10 ng/mL) Added to Liquid Orange Extract (Left: Rxi-5Sil MS; Right: Rtx-200 MS)

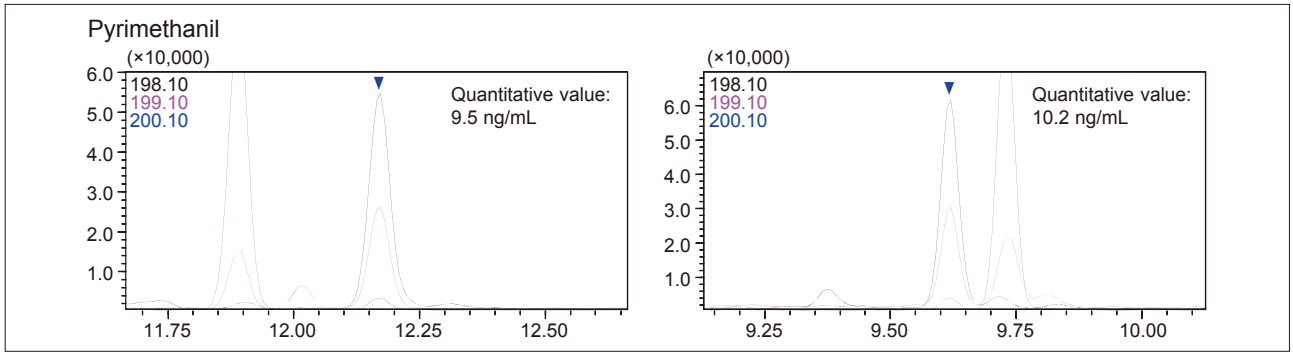


Fig. 4.6.4 Mass Chromatogram of Pyrimethanil (10 ng/mL) Added to Liquid Soya Beans Extract (Left: Rxi-5Sil MS; Right: Rtx-200MS)

4.7 Easy Screening for Residual Pesticides in Processed Foods (1) - GC/MS/MS

■Explanation

The analysis of residual pesticides in processed foods using GC-MS/MS, which provides excellent selectivity and sensitivity, has become a focus of attention. Before starting GC-MS/MS measurements, it is necessary to optimize MRM transitions (precursor ions and product ions) and collision energies (CE) for each pesticide measured, which is extremely labor intensive. Furthermore, in order to calculate quantitative values, it is necessary to prepare standard samples and create calibration curves. The Quick-DB database contains the optimal MRM conditions (MRM transitions and CE), mass spectra, retention indices, calibration curves and other information. This enables the semi-quantitative analysis of pesticides without using standard samples. Pesticide surrogates are used as the internal standard substances for calibration curves. Favorable quantitative accuracy is achieved by selecting the surrogates suited to each pesticide. In analyzing residual pesticides in processed foods, which contain a number of contaminants, separating the pesticides from the contaminants can be impossible, even with GC-MS/MS. In this case, an effective approach to separating and detecting the pesticides is to perform the analysis with two columns respectively, which differ in their separation patterns. The information registered in Quick-DB is also compatible with analysis using two different columns for residual pesticides in processed foods. In addition, if the Twin Line MS system is used, the two columns can be attached to the MS unit simultaneously, so data can be sampled from the different columns smoothly, without compromising the MS vacuum. Here we report on the results of applying Quick-DB and the Twin Line MS system to the analysis of residual pesticides in curry.

■Experimental

Using the Restek Q-sep™, commercially-available retort-pouch curry was pretreated via the QuEChERS method. The sample solution obtained was spiked with 230 standard pesticide samples at a concentration of 10 ng/mL. The pesticide-spiked samples were then subjected to Scan/MRM analysis under the analysis conditions registered in Quick-DB. The two columns indicated in Analytical Conditions were installed to a single GC-MS with the Twin Line MS system. The retention times for the pesticide components were estimated based on the analysis results for the n-alkane standard sample.

■Analytical Conditions

Instrument : GCMS-TQ8030 (Twin Line MS System)
Column 1 : Rxi-5Sil MS (30 m L., 0.25 mm I.D., df = 0.25 µm) (Restek Corporation, P/N: 13623)
Column 2 : Rtx-200MS (30 m L., 0.25 mm I.D., df = 0.25 µm) (Restek Corporation, P/N: 15623)
Glass Insert : Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek Corporation, P/N: 567366)

[GC]

Injection Temp.	: 250 °C
Column Temp.	: 60 °C (1 min) - (25 °C/min) - 160 °C - (4 °C/min) - 240 °C - (10 °C/min) - 290 °C (11 min)
Injection Mode	: Splitless
High Pressure Injection	: 250 kPa (1.5 min)
Carrier Gas Control	: Linear velocity (40.0 cm/sec)
Injection Volume	: 2 µL

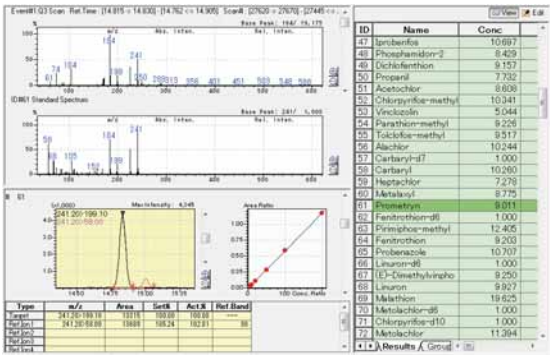
[MS]

Interface Temp.	: 300 °C
Ion Source Temp.	: 200 °C
Solvent Elution Time	: 1.5 min
Measurement Mode	: FAAST (Scan/MRM simultaneous measurement)
Scan Event Time	: 0.15 sec
Scan Mass Range	: *m/z* 50 to 330
Scan Speed	: 5,000 u/sec

■Analysis Results

The liquid food extract spiked with pesticides was analyzed, and data processing was performed with Quick-DB. The analysis results are shown in Fig. 4.7.1. When semi-quantitative analysis was performed using the calibration curves registered in Quick-DB, favorable semi-quantitative values were obtained, close to the additive concentration of 10 ng/mL for many of the components. To evaluate the quantitative accuracy for this analysis method, ratios were calculated for the semi-quantitative values with respect to the additive concentration. Then the pesticides were classified into those with a ratio under 50 %, 50 % to 200 %, and over 200 %, to find the distribution. The results are shown in Fig. 4.7.2. A significant 83 % of components had a semi-quantitative value 50 % to 200 % that of the concentration of the standard pesticide samples added. From this, it is evident that semi-quantitative analysis can be performed with high accuracy.

4.7 Easy Screening for Residual Pesticides in Processed Foods (2) - GC/MS/MS



As a result of calculation of the semi-quantitative values using the calibration curves preregistered in Quick-DB, favorable quantitative values were obtained. (*The concentration of the internal standard is indicated as 1 ng/mL.)

Fig. 4.7.1 Analysis Results for the Pesticide-Spiked Samples (10 ng/mL concentration)

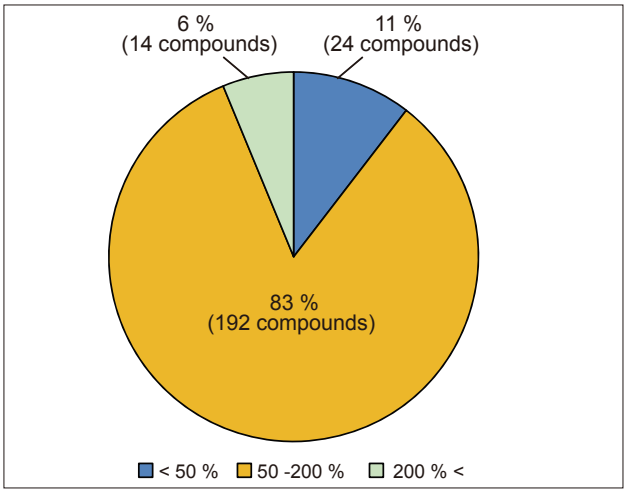


Fig. 4.7.2 Percentage Distribution of Semi-Quantitative Values with Respect to the Additive Concentration

In the analysis of residual pesticides in foods, when pesticide peaks are detected, it is necessary to check whether contaminants have been misidentified as pesticides, and whether contaminant overlap has inflated the size of the quantitative values. One confirmation method is to analyze the samples with columns with different separation patterns, and then check that essentially the same quantitative values are obtained for the pesticides detected in the respective columns. As an example, Fig. 4.7.3 shows the analysis results for dimethoate. With the Rxi-5Sil MS, there was an impact from contaminants, but with the Rtx-200MS, there was not. Semi-quantitative value obtained from the calibration curves registered in Quick-DB was favorable, 9.6 ng/mL, for the use of the Rtx-200MS column. In this way, even for pesticides of which separation from contaminants is difficult, separation is possible if using columns with different separation patterns, enabling highly reliable semi-quantitative analysis.

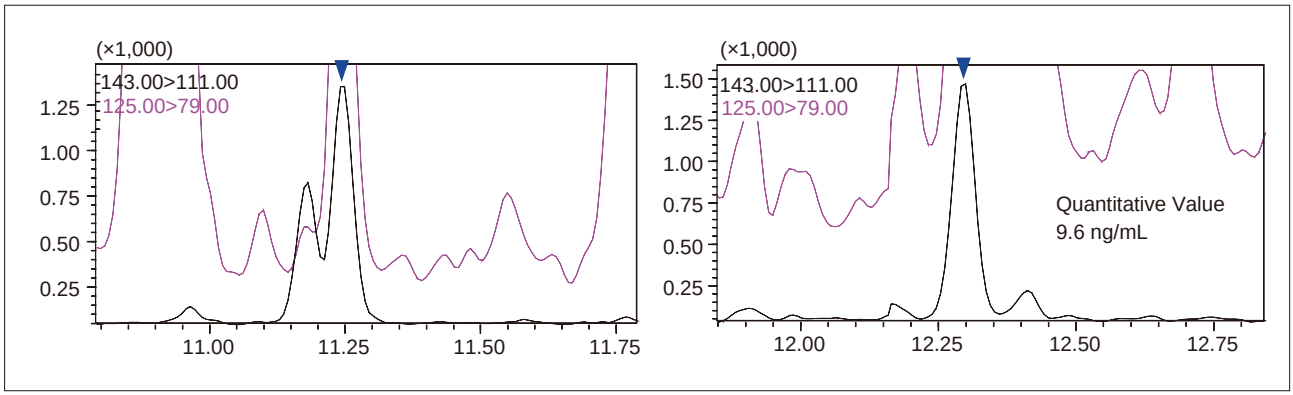


Fig. 4.7.3 Chromatograms for Liquid Curry Extract, Spiked with Dimethoate (10 ng/mL Concentration)
(Left: Rxi-5Sil MS; Right: Rtx-200MS)

High-accuracy semi-quantitative analysis was achieved quickly and easily, by attaching two columns to the GCMS-TQ8030 utilizing the Twin Line MS system, and then screening for residual pesticides in processed foods using Quick-DB.

4.8 High Throughput LC-MS/MS Analysis of Carbendazim in Orange Juice (1) - LC/MS/MS

■Explanation

A new high throughput LC-MSMS method was developed to facilitate increased testing for Carbendazim at low ppb levels. Carbendazim is a broad spectrum fungicide used in cereal and fruit crops. Although its use is banned in the US, there are a number of countries that still legally use Carbendazim to help control molds such as black spot. Recently, orange juice imported from Brazil has tested positive for Carbendazim. Since a tolerance level has not been established by the FDA, carbendazim is considered an unlawful pesticide residue. A number of commercial orange juices were tested from the local area and found to contain Carbendazim in low ppb levels. A novel high throughput method LC-MSMS method was developed that allows the analysis of Carbendazim to be completed in less than one minute to facilitate the testing of multiple samples.

■Experimental

Orange juice samples were diluted 40 to 50x with water and centrifuged at 3,000 rpm for one minute before analysis. Isocratic and gradient reversed phase high speed methods were developed for the analysis of Carbendazim on a 2.0 × 50 mm 1.6 micron column coupled to a tandem quadrupole mass spectrometer. A high throughput injector that features a seven second injection time was used to help speed the sample throughput.

Three commercial brands of orange juice tested positive for Carbendazim at low ppb levels. An organic brand of orange juice did not contain any detectable levels of Carbendazim and was used to prepare the matrix matched calibration curves. Good linearity was obtained from low ppb levels up to a 1 ppm concentration. A blank injected after the 1 ppm standard showed no carryover. The use of UHPLC with a high speed injector allowed an analysis to be completed within a one minute timeframe.

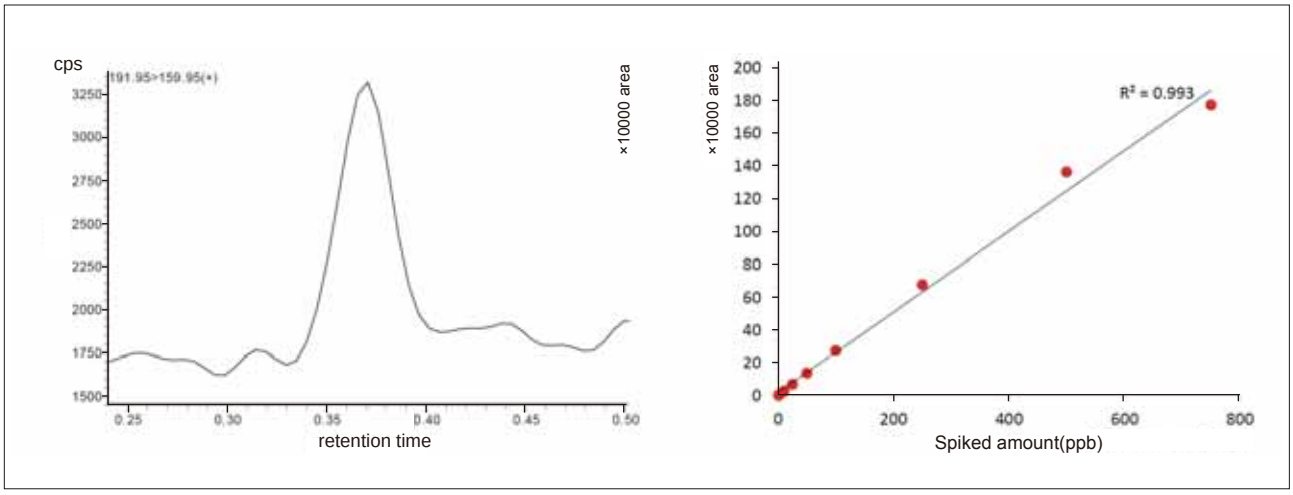


Fig. 4.8.1 Chromatogram of Orange Juice Spiked with 1 ppb Carbendazim and Calibration Curve

4.8 High Throughput LC-MS/MS Analysis of Carbendazim in Orange Juice (2) - LC/MS/MS

Table 4.8.1 shows the levels of carbendazim measured in different orange juice brands. Fig. 4.8.2 is the chromatogram of an orange juice matrix blank injected after 1 ppm standard. Fig. 4.8.3 and Fig. 4.8.4 are representative chromatograms of two brands of orange juice. The MM brand contained 8.1 ppb carbendazim, while none was detected in the certified organic orange juice.

Table 4.8.1 Levels of Carbendazim

Brand	Measured Carbendazim
HT *	ND
OV **	ND
GV	1.0 ppb
MM	8.1 ppb
T1	4.5 ppb
T2	1.1 ppb

* Generic store brand
** Organic certified

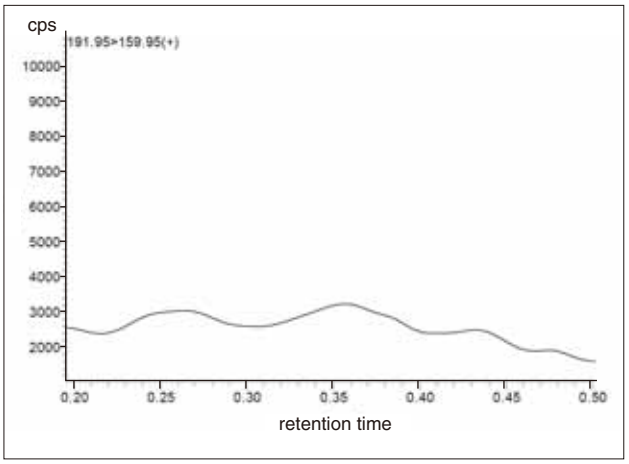


Fig. 4.8.2 Chromatogram of Orange Juice Matrix Blank

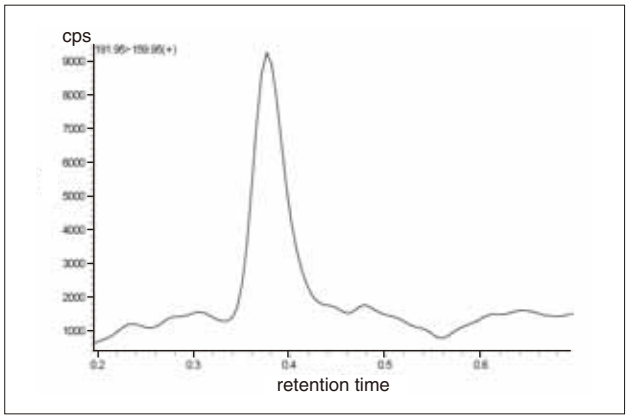


Fig. 4.8.3 Chromatogram of Brand MM Orange Juice

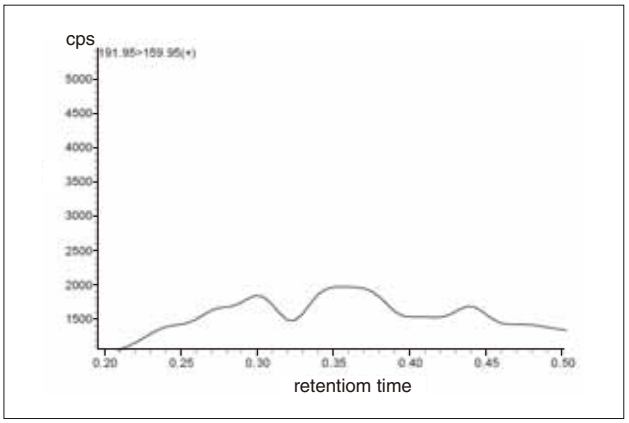
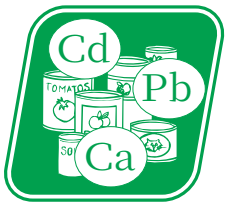


Fig. 4.8.4 Chromatogram of Certified Organic Orange Juice

Conclusion

Carbendazim was detected in four commercial brands of orange juice at low ppb levels. Levels found were below FDA limits for a positive test. The calibration curve was linear up to 500 ppb, and the method was sensitive enough to detect carbendazim below the most stringent regulatory threshold. No carryover was detected and the method run time was under one minute, thanks to the extremely high speed autosampler which completed each injection cycle in just 7 seconds. Sample preparation only required dilution and filtration for rapid, efficient analysis.



5. Inorganic Metals

5.1 Measurement of Lead in Sugar (1) - AA

■Explanation

The Japanese pharmacopeia specifies that analysis of lead content in refined white sugar be conducted by the electrothermal atomization (furnace) method. Since high sensitivity sample analysis is possible even at micro levels, this method is effective for trace level analysis even for toxic elements other than lead.
Here we introduce an example of such an analysis based on the method specified in the Japanese pharmacopeia.

■Pretreatment

The sample used for the analysis consisted of commercially available granulated sugar. Since atomic absorption spectrometry requires that the sample be in solution in order to conduct measurement, pretreatment of the refined sugar is necessary. Here we used a high pressure acid digestion vessel as indicated in the pretreatment procedure prescribed in the pharmacopeia. The high pressure acid digestion vessel consists of an internal PTFE vessel and an external metal or ceramic high pressure vessel. In the actual preparation, 0.050 g of sample was first accurately weighed into the internal PTFE vessel. To this, 0.5 mL of nitric acid was added for measurement of the toxic metals. The vessel was then placed in the external high pressure vessel, and this was heated for 5 hours in a 150 °C thermostatic chamber. After cooling, purified water was accurately added to bring the volume to 5 mL, and this was used as the measurement solution. In addition, a blank solution was prepared by adding purified water to 0.5 mL of the above-mentioned nitric acid to bring the volume to 5 mL.

■Analytical Method and Conditions

Quantitation was conducted by the standard addition method. The injection volumes of the prepared measurement solution, dilution-blank solution and Pb standard solution (20 ppb) were adjusted respectively using the autosampler. Tables 5.1.1 – 3 indicate the instrument used for the analyses and the main analytical conditions.

Table 5.1.1 Instrument and Optics Parameters

Instrument	Atomic absorption spectrophotometer unit: AA-7000 Atomizer unit: GFA-7000
Analysis wavelength	283.3 nm
Slit width	0.7 nm
Current	10 mA
Lamp mode	BGC-D2

Table 5.1.2 Furnace Program

	Temperature (˚C)	Time (s)	Mode	Sensitivity	Gas Flowrate (L/min)
1	60	3	RAMP	REGULAR	0.10
2	120	20	RAMP	REGULAR	0.10
3	250	10	RAMP	REGULAR	0.10
4	600	10	RAMP	REGULAR	1.00
5	600	10	STEP	REGULAR	1.00
6	600	3	STEP	HIGH	0.00
7*	2200	3	STEP	HIGH	0.00
8	2500	2	STEP	REGULAR	1.00

7* Atomization stage
Graphite tube: Pyrolysis tube

Table 5.1.3 Autosampler Standard Addition Parameter Settings

Addition Concentration	Sample	Diluent	Pb: 10 ppb	Total
0 ppb	14 µL	6 µL	0 µL	20 µL
1 ppb	14 µL	4 µL	2 µL	20 µL
2 ppb	14 µL	2 µL	4 µL	20 µL
3 ppb	14 µL	0 µL	6 µL	20 µL

5.1 Measurement of Lead in Sugar (2) - AA

■Results

The measurement results were under the quantitation limit. Fig. 5.1.1 shows an overlay of some typical peak profiles, and Fig. 5.1.2 shows the calibration curve using the blank solution. The quantitation limit in this measurement is 0.2 ppb for the concentration in aqueous solution, which converts to 0.03 ppm in the solid sample. This easily satisfies the criterion value of 0.5 ppm.

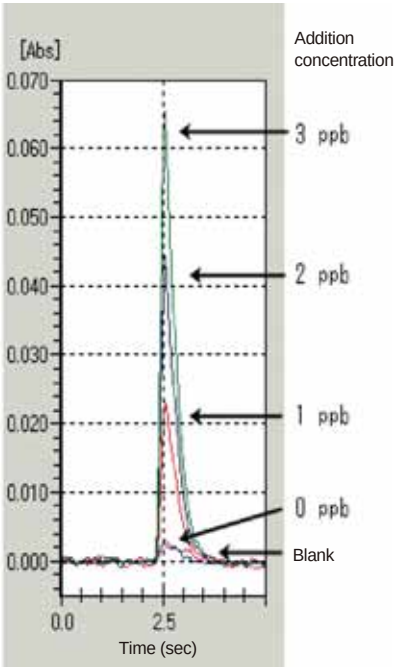


Fig. 5.1.1 Peak Profiles (partial)

In addition, Pb was added to the sample solution to bring the Pb concentration of the solution to 1.3 ppb (0.13 ppm in solid), and this was also measured as a sample. The results are shown in Fig. 5.1.3 and Table 5.1.4. The value was 0.13 ppm in the solid, indicating good correlation. The 1.43 dilution factor in Table 5.1.4 was calculated from the total injection volume of 20 µL with respect to the sample injection volume of 14 µL (20/14).

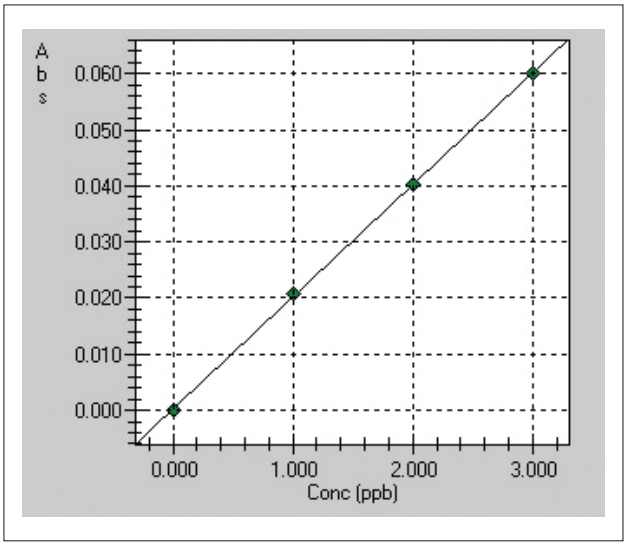


Fig. 5.1.2 Calibration Curve of Blank Solution

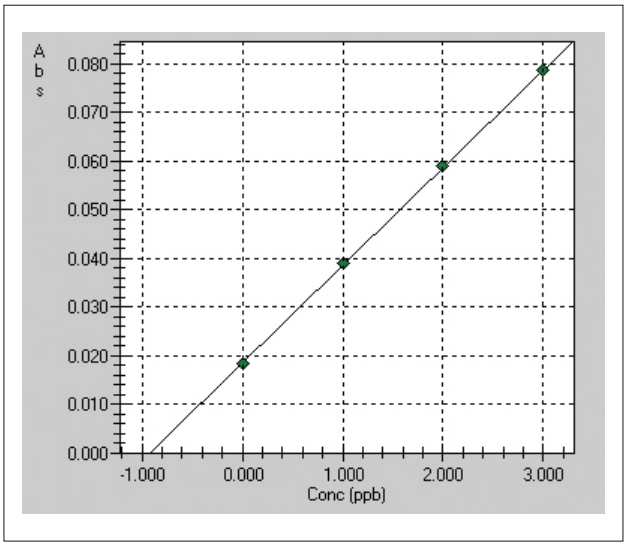


Fig. 5.1.3 Calibration Curve Using Sample Addition

Table 5.1.4 Sample Addition Results and Concentration Computation

Sample ID	Conc. Setting (ppb)	Conc. (ppb)	Abs.	Sample Amt.	Total Volume	Dilution Factor	Coefficient	Actual Conc.	Actual Conc. Unit	%RSD	SD
Blank			0.0023							2.47	0.0001
Added Conc: 0 ppb	0.0000		0.0184							3.19	0.0006
Added Conc: 1 ppb	1.0000		0.0389							1.12	0.0004
Added Conc: 2 ppb	2.0000		0.0589							0.87	0.0005
Added Conc: 3 ppb	3.0000		0.0787							1.03	0.0008
Added Sample		0.9253		0.0500	5.00	1.43	0.001	0.13	ppm		

5.2 Measurement of Minerals in Dietary Supplements (1) - AA

■Explanation

Recently, the development and sales of a variety of dietary supplements have increased dramatically against the background of rising public interest regarding health. Here we introduce the method of analysis of minerals in dietary supplements as specified in the Pharmacopoeia of the United States (USP 32), where the supplement market now stands at about three trillion yen (33 billion dollars). As one example, in the case of tablets of oil - and water- soluble vitamins with minerals, the sample preparation and measurement methods are specified for the quantitation of the minerals Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Se, and Zn, in which flame atomic absorption spectroscopy is used for conducting the quantitation.

■Sample Preparation

The sample preparation differs for (1) Ca, Cr, Cu, Fe, K, Mg, Mn, Zn and (2) Mo, Se in the above supplement. For the elements in group (1), at least 20 tablets are crushed and a quantity corresponding to 5 tablets are transferred to a porcelain crucible. After ashing at 550 °C in a muffle furnace, hydrochloric acid is added and the contents are heated to dissolve the residue. Adjust the final solution to 0.125 N hydrochloric acid. For the elements in group (2), at least 20 tablets are crushed, and a quantity corresponding to 1000 µg of the measurement element is weighed. This is decomposed using nitric acid and perchloric acid, and is finally brought to a fixed 2 % solution of ammonium chloride.

■Standard Concentrations of Elements

According to the USP, calibration curves are to be generated using standard solutions having the concentrations shown in Table 5.2.2, and quantitation is conducted using a calibration curve approximated by a straight line using a standard solution prepared for the concentration indicated in bold type in the Table. Examples of the target element calibration curves are shown in Fig. 5.2.1 – 10, but 0 µg/mL is not included in accordance with USP. In the case of Zn, since the high concentration of the standard solution causes curvature of the calibration curve at normal sensitivity, the angle of the burner was changed and measurement was conducted at lower sensitivity to improve the linearity.

■Measurement Conditions

The measurement wavelength, type of flame, and matrix modifier used are shown in Table 5.2.1. The N_2O-C_2H_2 flame was used for measurement of Ca and Mo, and the air-C_2H_2 flame was used for all the other elements. La was added as a matrix modifier for measurement of Ca and Mg, and ammonium chloride was added for measurement of Mo and Se.

Table 5.2.1 Measurement Conditions

Element	Wavelength	Flame	Matrix Modifier
Ca	422.7 nm	N_2O-C_2H_2	0.1 % La
Cr	357.9 nm	Air-C_2H_2	
Cu	324.7 nm	Air-C_2H_2	
Fe	248.3 nm	Air-C_2H_2	
K	766.5 nm	Air-C_2H_2	
Mg	285.2 nm	Air-C_2H_2	0.1 % La
Mn	279.5 nm	Air-C_2H_2	
Mo	313.0 nm	N_2O-C_2H_2	2 % Ammonium Chloride
Se	196.0 nm	Air-C_2H_2	2 % Ammonium Chloride
Zn	213.8 nm	Air-C_2H_2	

Table 5.2.2 Element Concentrations for Calibration Curves

Element	STD (µg/mL)						
	1	2	3	4	5	6	8
Mn		0.5	0.75	**1**		1.5	2
K	0.5	**1**	1.5	2	2.5		
Zn	0.5	1	1.5	**2**	2.5		
Cu	0.5	1		**2**		3	4
Cr		**1**		2		3	4
Fe		2		4	**5**	6	8
Mg		0.2	0.3	**0.4**	0.5	0.6	
Ca		1	1.5	**2**	2.5	3	
Mo	5	**10**			25		
Se	5	**10**			25		

5.2 Measurement of Minerals in Dietary Supplements (2) - AA

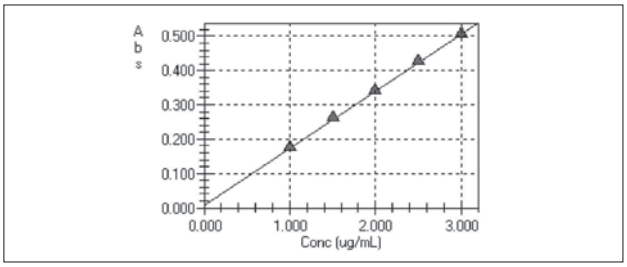


Fig. 5.2.1 Calibration Curve of Ca

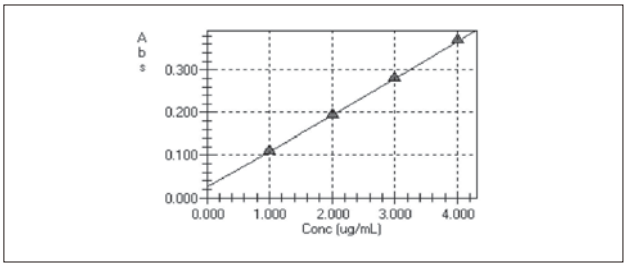


Fig. 5.2.2 Calibration Curve of Cr

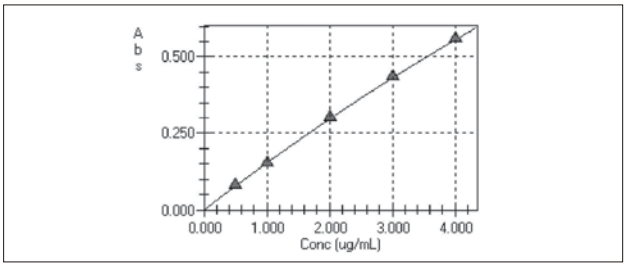


Fig. 5.2.3 Calibration Curve of Cu

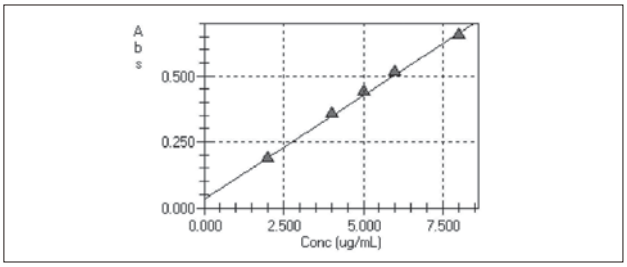


Fig. 5.2.4 Calibration Curve of Fe

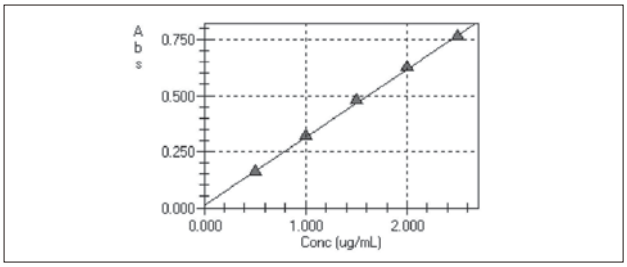


Fig. 5.2.5 Calibration Curve of K

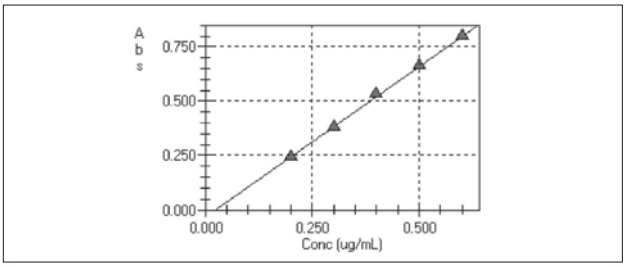


Fig. 5.2.6 Calibration Curve of Mg

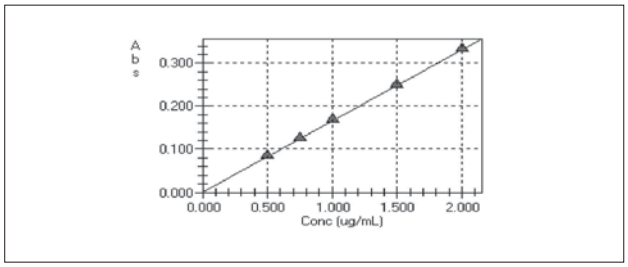


Fig. 5.2.7 Calibration Curve of Mn

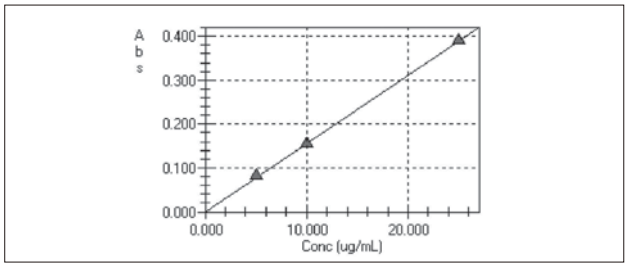


Fig. 5.2.8 Calibration Curve of Mo

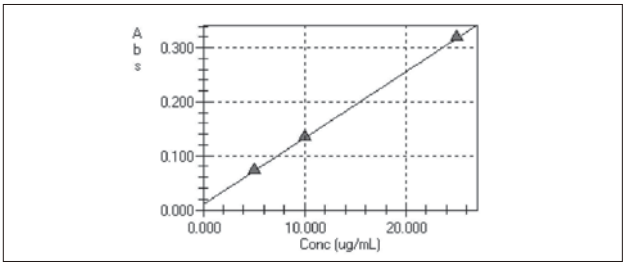


Fig. 5.2.9 Calibration Curve of Se

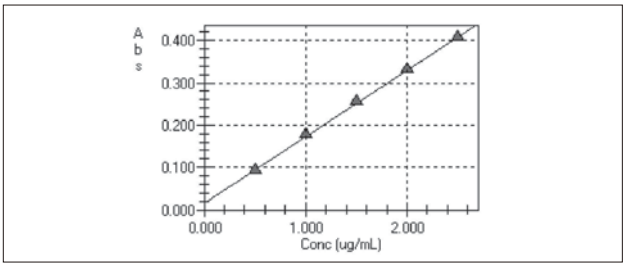


Fig. 5.2.10 Calibration Curve of Zn

5.3 Measurement of Cadmium and Lead in Food Additives (1) - AA

■Explanation

A food additive is defined in Japan's Food Sanitation Act as "an item to be used for the purpose of storage or processing of food, which is added to, mixed with, or diffused into food in any manner." Food additives are used for a variety of purposes, as, for example, preservatives, sweeteners, coloring agents, and stabilizers. Test methods and component standards have been established for many of these, and have been published as a food additives compendium titled "Japan's Specifications and Standards for Food Additives." One of the purity test items is heavy metal testing (in terms of lead content), for which the eighth edition of the compendium adopts a colorimetric method using Nessler cylinders. However, in the ninth edition, a different test method is under review, in which the element lead is handled individually. Here, we introduce an example of analysis of cadmium (Cd) and lead (Pb) in α-cyclodextrin (cyclic oligosaccharide), a substance used in functional foods, pharmaceuticals, cosmetics, etc. The analysis was conducted by electrothermal atomic absorption spectrometry (ETAAS) using the AA-7000 atomic absorption spectrophotometer.

■Sample Preparation

Sample digestion was conducted using the ETHOS One microwave sample preparation system (Milestone Srl). Compared to pretreatment using dry ashing or an open system such as wet digestion, microwave digestion permits quick digestion of the sample, making it unlikely that contamination or volatilization of the measurement element will occur. The digestion process flow is shown in Fig. 5.3.1. For validity assessment of the pretreatment and measurement, the same process was conducted on a sample spiked with standard solution prior to digestion. Preparation was conducted so that the spiked solid concentrations were 0.05 µg/g of Cd and 0.5 µg/g of Pb.

Transfer 0.5 g sample to digestion container.
↓
Add small amount of distilled water and 8 mL nitric acid, then mix.
↓
Seal container, set in ETHOS One, digest for approx. 1 hour.
↓
After cooling, transfer to a vessel, add distilled water to a volume of 50 mL.
↓
Measure the sample by electrothermal atomic absorption spectrometry.

Fig. 5.3.1 Flowchart of Sample Decomposition

■Analytical Method and Conditions

The standard solutions for atomic absorption analysis were prepared by diluting a 1000 mg/L standard solution to obtain 1 µg/L of cadmium and 10 µg/L of lead, respectively. The calibration curves were generated using an autosampler to adjust the injection volumes of standard solution in a stepwise manner. In addition, 5 µL of a palladium nitrate solution (50 mg/L palladium content) was added as a matrix modifier to all of the samples. The main conditions that were used for the spectrometer and atomization are shown in Tables 5.3.1 and 5.3.2.

Table 5.3.1 Optics Parameters

	Cd	Pb
Analytical wavelength	228.8 nm	283.3 nm
Slit width	0.7 nm	
Ignition mode	BGC-D2	

Table 5.3.2 Atomizing Parameters

	Cd	Pb
Ashing temperature	700 °C	800 °C
Atomizing temperature	2200 °C	
Standard solution concentration (ppb)	0.2, 0.5, 1.0	2.5, 5, 10
Tube type	Platform	
Sample injection volume	20 µL	
Matrix modifier	5 µL of 50 ppm palladium nitrate	None

With the microwave digestion method, since much of the acid that is added remains, it is not uncommon for the acid concentration in the sample solution to be more than 10 %. High acid concentration is a factor that can lead to a decrease in repeatability and sensitivity. The platform tube used for this measurement (see Fig. 5.3.2) is resistant to the effects of acidity and coexisting substances in the matrix because the sample is injected into a plate having a recess (platform) that is mounted in the tube and then uniformly heated by radiant heat from the outer wall.
Fig. 5.3.3 shows the changes in absorbance of a lead standard solution caused by varying the nitric acid concentration. A fairly constant absorbance was found to be obtained up to a nitric acid concentration of 20 %.

5.3 Measurement of Cadmium and Lead in Food Additives (2) - AA

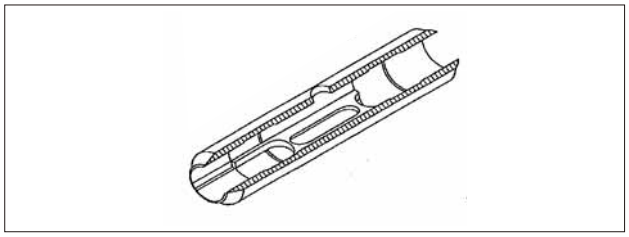


Fig. 5.3.2 Sectional View of Platform Tube

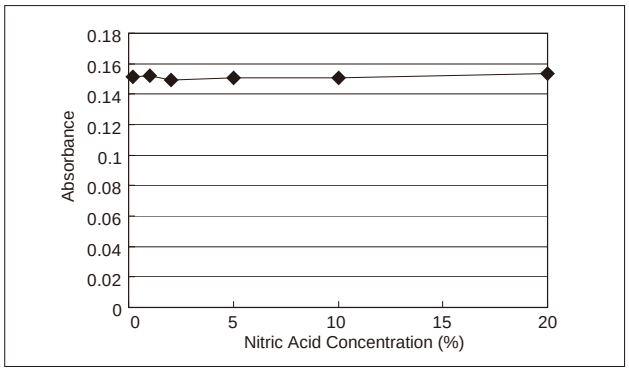


Fig. 5.3.3 Sensitivity Variation of Pb 10 µg/L Standard Solution Due to Changes in Nitric Acid Concentration When Using a Platform Tube

Results and Conclusion

The sample measurement results are shown in Table 5.3.3. Neither of the elements was detected in the sample. Calculation of the lower limit of quantitation as a concentration in a solid at an absorbance of 0.01 Abs yielded 0.003 µg/g for cadmium and 0.07 µg/g for lead. Good values were obtained in spike and recovery testing, and high-sensitivity analysis of heavy metals was possible using electrothermal atomic absorption spectrometry with a platform tube, without adverse effects from the acid concentration. The calibration curves are shown in Figs. 5.3.4 and 5.3.5, respectively, and the peak profiles are shown in Fig. 5.3.6. The AA-7000 Series features a lineup that includes not only dedicated instruments for the flame method and electrothermal method, but also a dual-use instrument that offers automatic switching of the atomization method, thereby supporting a wide range of application requirements. Regarding the α-cyclodextrin that was measured in this application, the eighth edition of the Specifications and Standards for Food Additives specifies a separate reference value for lead (1 µg/g or less). For pretreatment, after ashing 10 g of sample, nitric acid is added to bring the solution to a volume of 10 mL, and flame atomic absorption is specified as the measurement method. When the AA-7000 flame method is used to analyze the sample prepared in this manner, the expected detection limit of lead in a solid sample is about 0.2 µg/g.

Table 5.3.3 Measurement Results of Cd and Pb in α-Cyclodextrin

Element	Cd	Pb
Measured value	< 0.003 µg/g	< 0.07 µg/g
Spike and recovery rate	105 %	99 %

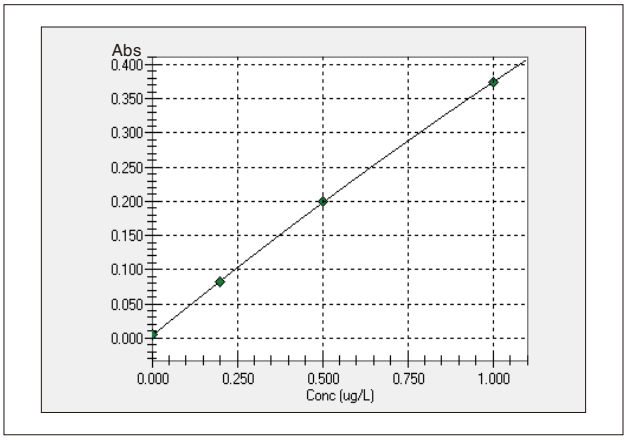


Fig. 5.3.4 Calibration Curve of Cd

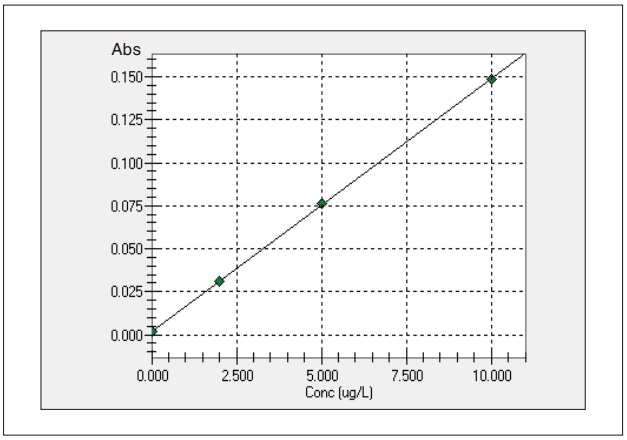


Fig. 5.3.5 Calibration Curve of Pb

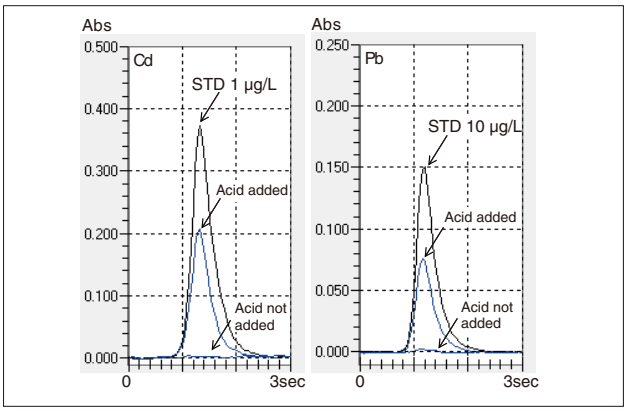


Fig. 5.3.6 Peak Profiles

5.4 Measurement of Heavy Metals (Cd, Pb) in Pet Food (1) - AA

■Explanation

In 2007, an incident occurred in which pet food was found to contain melamine that had been mixed in with the pet food in the country where it was produced. However, this was discovered only after many pet cats and dogs died as a result of melamine poisoning. As a result of this incident, the Japanese Ministry of the Environment and the Ministry of Agriculture, Forestry and Fisheries established a study group to address this situation, and in 2008, the Act on Ensuring of Safety of Pet Animals Feed was enacted. Based on this law, an ordinance pertaining to standards of pet animal feed components was promulgated (Amendment, September 1, 2011, Ministry of Agriculture, Forestry and Fisheries, Ministry of Environment, Ordinance No. 3), and has been in effect since March 1, 2012. Also cited in this ordinance are mycotoxins, organochlorine compounds, and three heavy metal elements, cadmium, lead and arsenic. The concentrations of heavy metals in pet foods for sale are required to meet the criteria of Table 5.4.1, assuming a 10 % moisture content.

Table 5.4.1 Regulated Values for Heavy Metals in Pet Food

Cd	1 ppm or less
Pb	3 ppm or less
As	15 ppm or less

Here, we introduce an example of analysis of Cd and Pb in pet food using the AA-7000 Atomic Absorption Spectrophotometer.

■Sample Preparation

The sample consisted of dry pet food that was finely ground in a food processor. Sample digestion for electrothermal atomic absorption was conducted using the ETHOS One microwave sample preparation system (Milestone Srl). The digestion process flow is shown in Fig. 5.4.1. For validity assessment, the same preparation process was conducted on a sample spiked with standard solution prior to digestion. Preparation was conducted so that the spiked solid concentrations were 0.5 ppm of Cd and 1 ppm of Pb.

Transfer 0.5 g sample to digestion container.
↓
Add small amount of distilled water and 8 mL nitric acid, then mix.
↓
Seal container, set in ETHOS One, digest for approx. 1 hour.
↓
After cooling, transfer to a vessel, add distilled water to a volume to 50 mL.
↓
Conduct measurement by electrothermal atomic absorption.

Fig. 5.4.1 Flow Chart of Sample Digestion for ETAAS (Electrothermal Atomic Absorption Spectrometry)

Processing for flame measurement was conducted according to the Test Method for Pet Animal Feed (established by the Food and Agricultural Materials Inspection Center, the Ministry of Agriculture, Forestry and Fisheries of Japan, No. 1764, September 1, 2009). Fig. 5.4.2 shows the flow chart for sample digestion, and Fig. 5.4.3 the flow chart for solvent extraction for Flame AA, respectively. The solvent extraction process is intended for removal of coexisting substances and the concentration of analyte elements.

Transfer 10 g of sample to 100 mL tall beaker.
↓
Ashing by electric furnace (up to 500 °C maximum)
↓
Let cool, then add 10 mL HCl, and water to about 30 mL.
↓
Heat for several minutes.
↓
Let cool, adjust volume to 100 mL.
↓
Collect filtrate (six filter paper) and use as sample.

Fig. 5.4.2 Flow Chart of Sample Digestion for Flame AA

Transfer 30 mL sample solution to separatory funnel containing 14 mL phosphoric acid.
↓
Add 5 mL potassium iodide solution (68 w/v%) and purified water to bring volume to about 50 mL.
↓
Gently shake to mix, and let stand 5 minutes.
↓
Accurately add 10 mL MIBK, and after shaking vigorously, let stand.
↓
Collect MIBK layer in test tube and measure by Flame AA.

Fig. 5.4.3 Flow Chart of Sample Extraction for Flame AA

■Analytical Method and Conditions

The standard solution for electrothermal measurement by atomic absorption analysis was prepared by diluting a 1000 mg/L standard solution to obtain 2 ppb (µg/L) of Cd and 20 ppb of Pb (µg/L). A calibration curve was generated using an autosampler to prepare stepwise increasing injection volumes. In addition, 5 µL of a palladium nitrate solution (100 ppm (mg/L) palladium content) was added as a matrix modifier to all of the samples. The standard solution used for the flame AA method was prepared using solvent extraction similarly as for the sample solution. The main conditions that were used for the spectrometer and atomization are shown in Tables 5.4.2 - 4.

5.4 Measurement of Heavy Metals (Cd, Pb) in Pet Food (2) - AA

Table 5.4.2 Optics Parameters

	Cd	Pb
Analytical wavelength	228.8 nm	283.3 nm
Slit width	0.7 nm	
Ignition mode	BGC-D2	

Table 5.4.3 Atomizing Parameters for ETAAS

	Cd	Pb
Ashing temperature	600 °C	900 °C
Atomizing temperature	2200 °C	2400 °C
Standard solution oncentration (ppb)	0.5, 1.0, 2.0	5, 10, 20
Tube type	Platform	
Matrix modifier	5 µL of 100 ppm palladium nitrate	

Table 5.4.4 Atomizing Parameters for Flame AA

	Cd	Pb
Flame type	Air – Acetylene	
Acetylene flowrate	0.8 L/min	
Standard solution concentration (ppm)	0.05, 0.10, 0.20	0.25, 0.50, 1.00

The standard solution concentration refers to the concentration in the solvent.

Results and Conclusion

Table 5.4.5 shows the sample measurement results. The values were converted to indicate the concentrations in the solid sample. Table 5.4.6 shows the lower limits of quantitation. The calculated concentrations in the solid sample are based on the absorbance values of 0.01 Abs and 0.004 Abs obtained using the Electrothermal and Flame methods, respectively. Fig. 5.4.4 and Fig. 5.4.5 show the calibration curves and measurement solution peak profiles by the Electrothermal method.

Table 5.4.5 Measurement Results for Cd and Pb in Dog Food

Element	Cd	Pb
Reference value	1 ppm or less	3 ppm or less
Electrothermal method	0.19 ppm (94 %)	0.26 ppm (106 %)
Flame method	0.20 ppm	< 0.3 ppm

Values in parentheses indicate spike/recovery rate.

Table 5.4.6 Limit of Quantitation of Cd and Pb

Element	Cd	Pb
Electrothermal method	0.003 ppm	0.07 ppm
Flame method	0.01 ppm	0.3 ppm

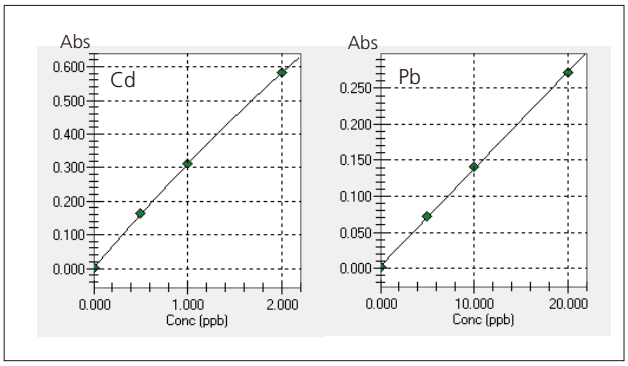


Fig. 5.4.4 Calibration Curves by ETAAS

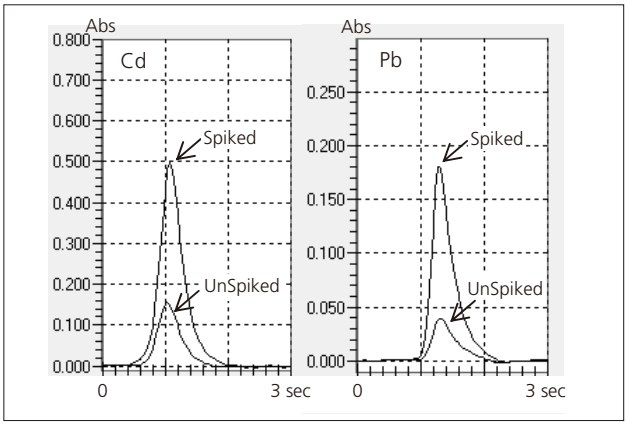


Fig. 5.4.5 Peak Profiles of Sample and Spiked Sample by ETAAS

The measurement results obtained using microwave digestion – electrothermal atomic absorption were in good agreement with the measurement results obtained by the method prescribed in the Test Method for Pet Animal Feed, and excellent spike and recovery results were also obtained. Further, compared to the dry ashing – solvent extraction pretreatment operations, preparation of the measurement solution by microwave digestion can be accomplished more quickly, and combined with the electrothermal absorption method using the AA-7000, heavy metals could be analyzed with high sensitivity. Not only does the AA-7000 support analysis by the flame and electrothermal methods, the lineup includes a model that permits automated switching between both atomization methods to accommodate a wide range of requirements.

5.5 Measurement of Cadmium in Brown Rice (1) - AA

■Explanation

The standard for restricting the amount of cadmium in rice was strengthened in Japan from a maximum of 10 mg/kg to a maximum of 0.4 mg/kg as specified in the Food Sanitation Law according to the Ministry of Health, Labour and Welfare notification No. 183 (notification of April 8, 2010). This standard was applied on February 28, 2011. Although this restriction previously applied only to brown rice, it now includes polished rice. Here we present an example of analysis of cadmium in a standard sample (NIES No. 10-a) of brown rice by the high-sensitivity electrothermal atomic absorption method using the AA-7000. For an example of cadmium analysis in rice by the flame method and by the ICP emission spectrometry, refer to Reference 1 and Reference 2, respectively.

■Sample Preparation

After weighing out 1 g of sample into a beaker, nitric acid and hydrogen peroxide were added and thermal decomposition was conducted. The decomposition flow is shown in Fig. 5.5.1. This pretreatment process was repeated 6 times on the same sample to evaluate the repeatability of the preparation process.

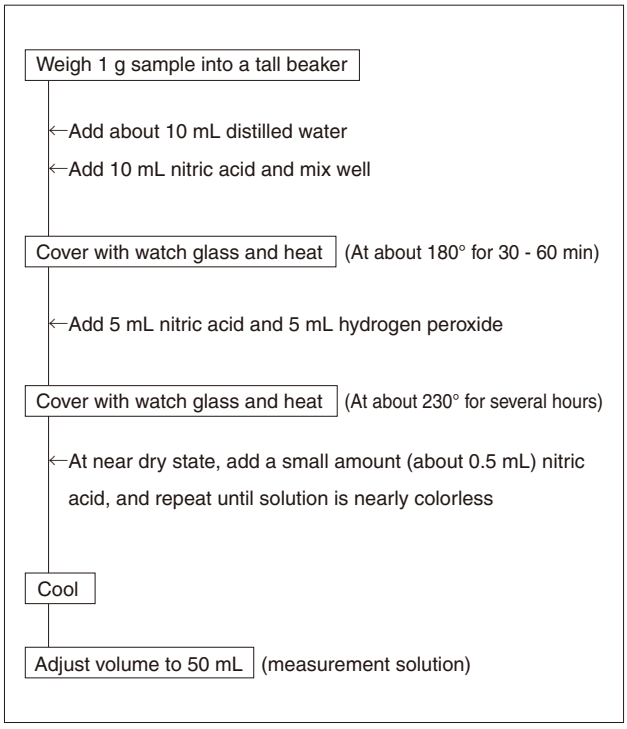


Fig. 5.5.1 Sample Decomposition Flow Chart

■Analytical Method and Conditions

Measurement was conducted using the calibration curve method. A 1000 mg/L standard solution for atomic spectrometry was diluted to prepare a 2 µg/L standard solution for the measurement. The calibration curve was generated by changing the injection volume of this standard solution in stepwise fashion using an autosampler. In addition, 5 µL of palladium nitrate solution (100 mg/L Pd) was added to each of the samples, including the standard solution, as a matrix modifier.

The main measurement conditions are shown in Tables 5.5.1 and 5.5.2.

Table 5.5.1 Optics Parameters

Analysis wavelength	228.8 nm
Slit width	0.7 nm
Current values	Low 8 mA High 100 mA
Lamp mode	BGC-SR

Table 5.5.2 Atomizing Parameters

Temperature Program	No.	Temperature (ºC)	Time (sec)	Heating Mode	Gas Flowrate (L/min)
	1	60	3	RAMP	0.1
	2	150	15	RAMP	0.1
	3	250	10	RAMP	0.1
	4	600	10	RAMP	0.2
	5	600	15	STEP	0.2
	6	600	3	STEP	0.0
	7	2200	3	STEP	0.0
	8	2400	2	STEP	1.0
	Atomization stage is No. 7				
Sample injection volume	20 µL				
Matrix modifier	Palladium nitrate solution (palladium concentration 100 ppm) 5 µL				
Tube type	High density graphite tube				
Signal processing	Peak height				

5.5 Measurement of Cadmium in Brown Rice (2) - AA

■Measurement Results

Table 5.5.3 shows the sample measurement results. At a low concentration of 1/10 the standard value, excellent results were obtained for both concentration and repeatability.

Fig. 5.5.2 and Fig. 5.5.3 show the calibration curve and segments of the standard solution peak profiles, respectively.

Fig. 5.5.4 shows the peak profiles of the respective samples.

Table 5.5.3 Measurement Results of Cd in Brown Rice

	NIES No. 10-a (Guaranteed value 0.023 ± 0.003 mg/kg)
Process 1	0.024 mg/kg
Process 2	0.024 mg/kg
Process 3	0.023 mg/kg
Process 4	0.023 mg/kg
Process 5	0.024 mg/kg
Process 6	0.023 mg/kg
Average	0.024 mg/kg
Standard deviation	0.0004 mg/kg
Relative standard deviation (%RSD)	1.7 %

Each value is an average of n=2.

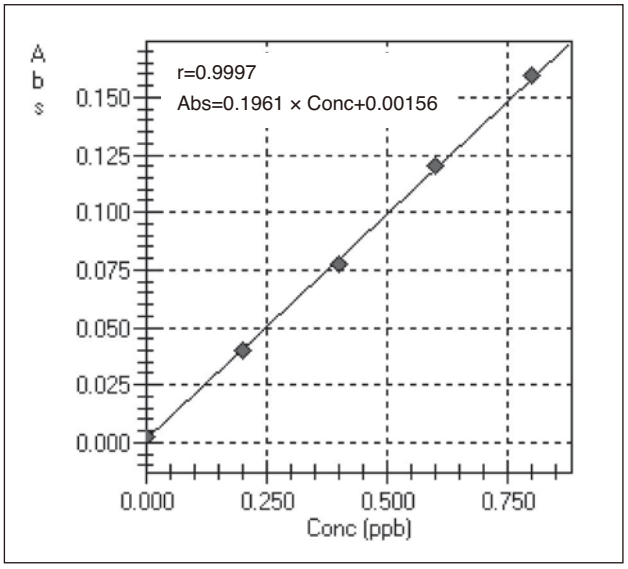


Fig. 5.5.2 Calibration Curve

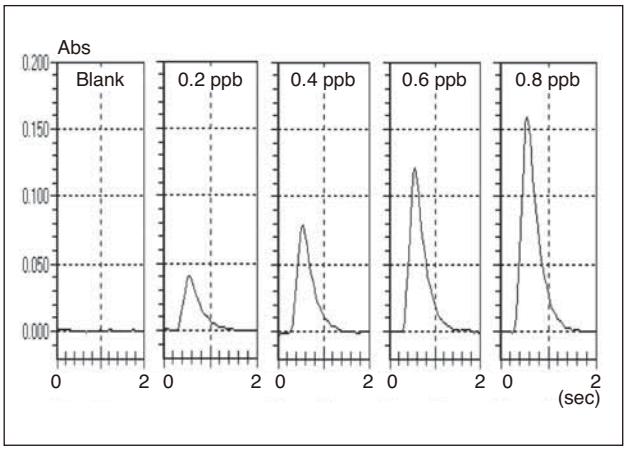


Fig. 5.5.3 Peak Profiles of Standards

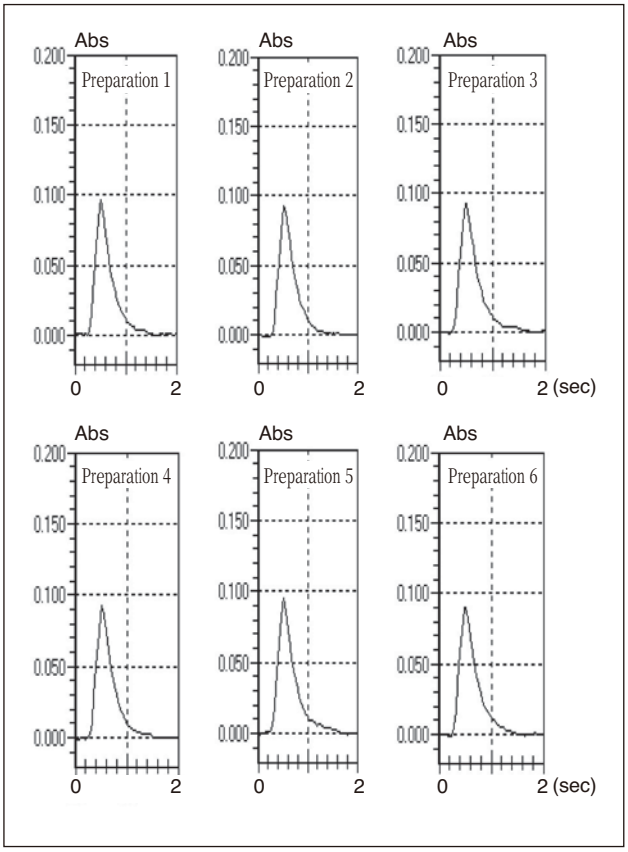


Fig. 5.5.4 Peak Profiles of Samples

[References]

1) Shimadzu Application News No. A427 "Determination of Cadmium in Brown Rice by Flame Atomic Absorption" (2010)
2) Shimadzu Application News No. J87 "Multi-Element Simultaneous Determination of Nutrients as well as Hazardous Elements in Brown Rice by ICPE-9000" (2007)

6.1 Analysis of Diarrhetic Shellfish Poison (DSP) (1) - LC/MS

■Explanation

Diarrhetic Shellfish Poison (DSP) is a substance that accumulates in the digestive gland of the shellfish when they ingest poisonous planktons such as *Dinophysis fortii* or *Dinophysis acuminata*. When humans ingest shellfish affected by DSP, they suffer acute gastroenteritis accompanied by such symptoms as vomiting, diarrhea and stomachache. Although the amount ingested normally does not lead to death in humans, it is known that DSP does not decompose at normal household cooking temperatures. Typical DSP includes Okadaic acid (OA), Dinophysistoxin (DTX), Pectenotoxin (PTX), and Yessotoxin. Since they have complex structures and no suitable chromophore groups, their analysis is difficult. In Japan, DSP is officially analyzed by a bioassay method called the mouse unit method. However, HPLC analysis combined with fluorescence derivatization is also applicable.

Introduced here is an example of analysis of OA, DTX-1 and PTX-6 (Fig. 6.1.1) using LC/MS. The ESI mass spectra of the positive and negative ions of OA were shown in Fig. 6.1.2. In the negative ion ESI method, the deprotonated molecule $(M-H)^-$ is clearly detected. In the positive ion spectrum, the molecule with the attached sodium ion $(M+Na)^+$, as well as fragment ions where 1 - 4 water molecules have detached from the protonated molecule, are detected.

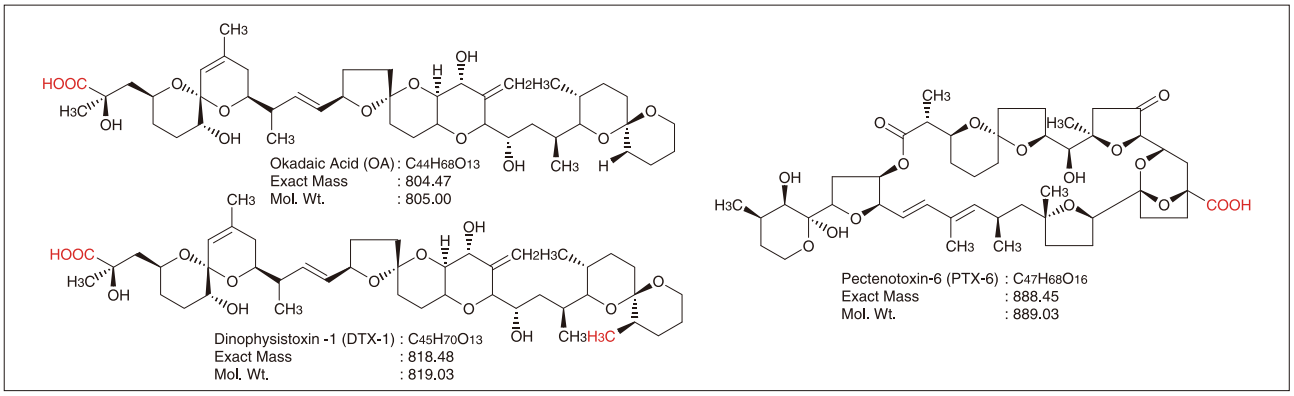


Fig. 6.1.1 Structures of Okadaic Aid (OA), Dinophysistoxin-1 (DTX-1) and Pectenotoxin-6 (PTX-6)

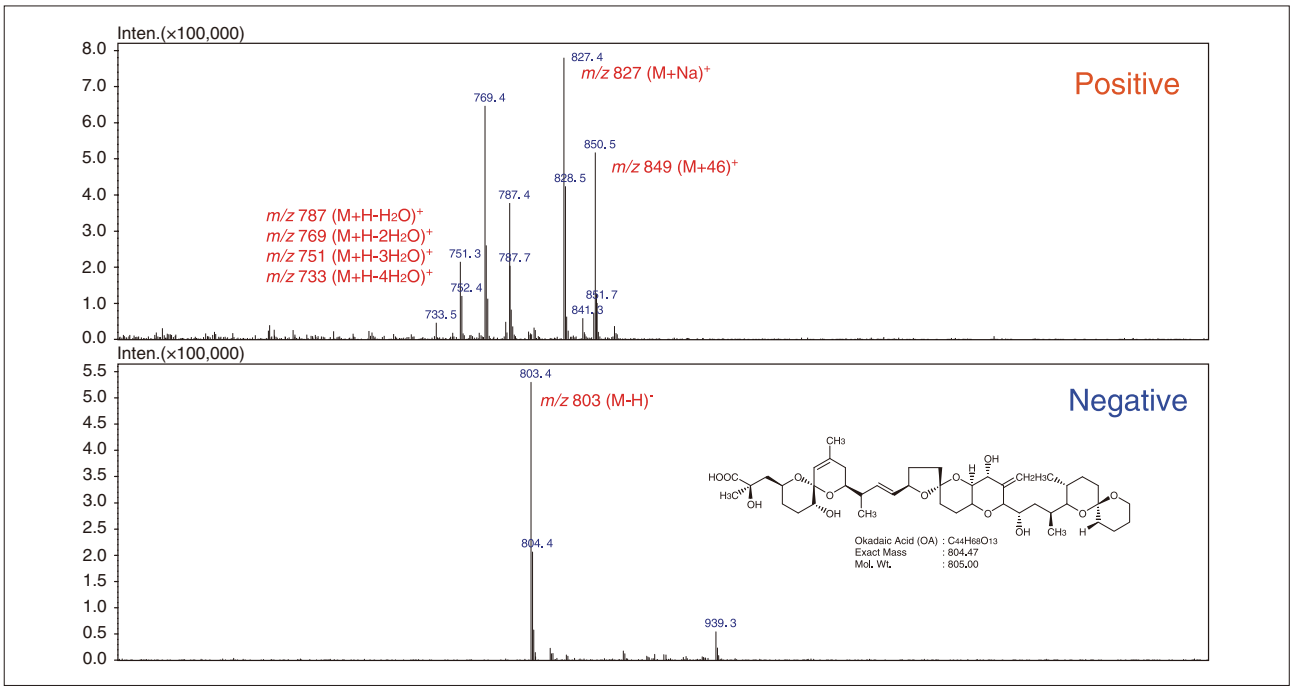


Fig. 6.1.2 Positive and Negative ESI Mass Spectra of Okadaic Aid (OA)

6.1 Analysis of Diarrhetic Shellfish Poison (DSP) (2) - LC/MS

The results of selected ion monitoring (SIM) analysis using *m/z* of the deprotonated molecule $(M-H)^-$ in the negative ion detection and *m/z* of the sodium ion-added molecule $(M+Na)^+$ in the positive ion detection yielded good linearity in the concentration range of 13-1625 ng/mL, with a quantitation limit of 13 ng/mL (excluding PTX-6 in negative ion detection). As for PTX-6, the sensitivity decreases to about one-fifth in the negative ion detection compared to the positive ion detection, and PTX has isomers that do not contain carboxylic acid. Although these factors make the spectrum comparatively complex, positive ion detection is more suitable when PTX needs to be analyzed.

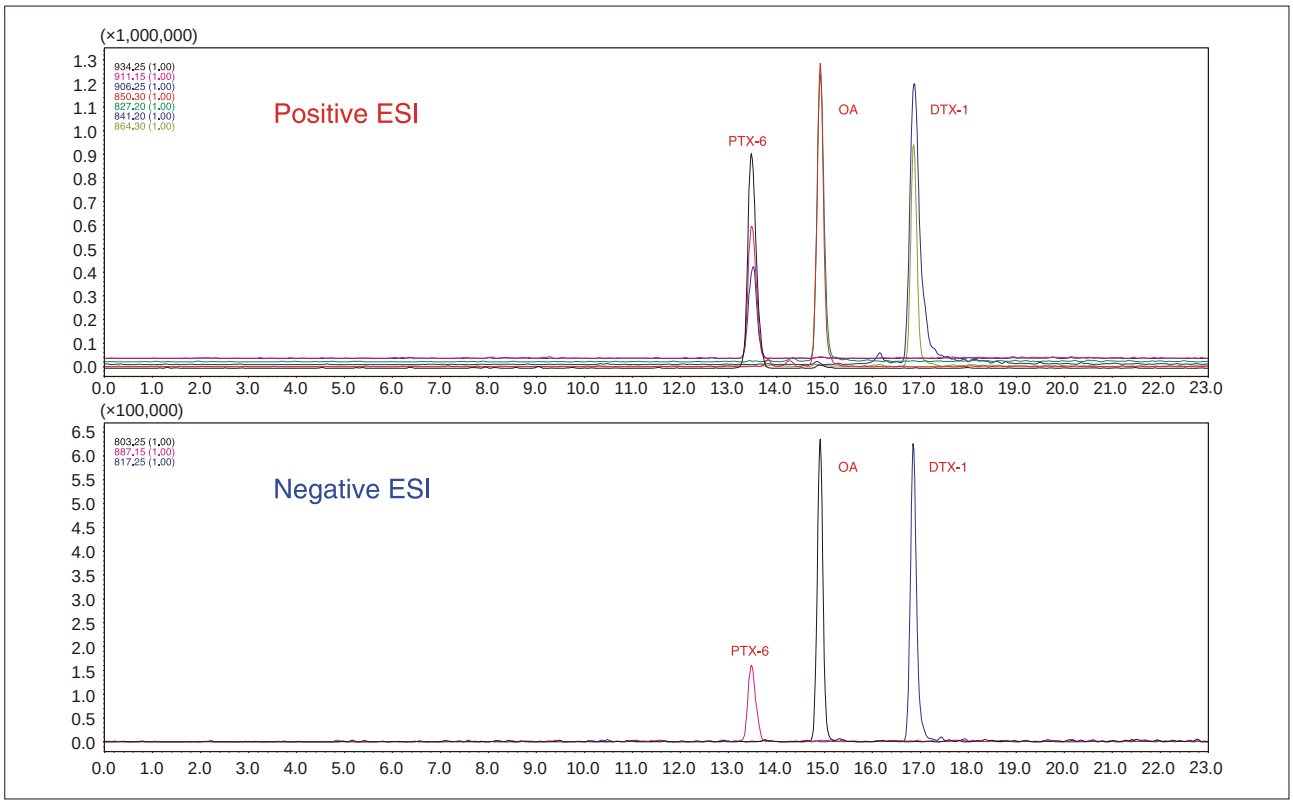


Fig. 6.1.3 SIM Chromatograms of DSP using Positive and Negative ESI

■Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 2.0 mm I.D.)	
Mobile Phase A	: 5 mmol/L Ammonium Acetate + 0.1 % Formic Acid-Water	
Mobile Phase B	: Acetonitrile	
	Gradient Elution Method	
Time Program	: B 10 % (0 min) → 90 % (15-20 min)	
Flowrate	: 0.2 mL/min	
Column Temp.	: 40 °C	
Injection Volume	: 2 µL	
Probe Voltage	: -3.5 kV (ESI-Negative Mode)	+ 4.5 kV (ESI-Positive Mode)
Nebulizer Gas Flow	: 1.5 L/min	←
Drying Gas Pressure	: 0.2 MPa	←
CDL Temp.	: 200 °C	←
Block Heater Temp.	: 200 °C	←
CDL & Q-array Voltage	: Using default values	←
SIM	: *m/z* 803.25, 887.15, 817.25 for Negative Mode	
	m/z 906.25, 911.15, 634.25, 850.30, 827.20, 864.30, 841.20 for Positive Mode	

6.2 High Speed Analysis of Aflatoxins in Food (1) - LC

■Explanation

Aflatoxins are mycotoxins that are extremely carcinogenic and acutely toxic, and because they are subject to food contamination monitoring, their measurement is routinely conducted by HPLC and other analytical methods. Here we introduce an example of ultra-high-speed analysis of aflatoxins in food using a combination of the Prominence RF-20Axs high-sensitivity fluorescence detector and the Nexera Ultra High Performance LC system, in which the aflatoxins B_1, B_2, G_1 and G_2 are analyzed using direct high-sensitivity fluorescence detection without conducting TFA derivatization.

■Analysis of Aflatoxins Standard Solution

Fig. 6.2.1 shows a chromatogram of a standard mixture of the 4 aflatoxins. For the analytical column, the Shim-pack XR-ODSII (100 mm L. × 3.0 mm I.D., 2.2 µm) was used. The concentrations of B_1 and G_1 were each 20 ng/L (20 ppt), and those of B_2 and G_2 were each 5 ng/L (5 ppt). The mixture was prepared in a solution of water / acetonitrile = 9/1 (v/v). Use of the RF-20Axs allowed high sensitivity detection of B_1 and G_1 without undergoing derivatization with TFA.

Although an injection volume of 8 µL was used in the analysis shown in Fig. 6.2.1, we investigated the peak shapes and separation performance using larger sample injection volumes for analyses at even lower trace levels. Fig. 6.2.2 shows the chromatograms of the same standard mixture of 4 aflatoxins used in the analysis of Fig. 6.2.1, but with injection volumes of 8 µL, 30 µL, and 50 µL, respectively. Even with a 50 µL injection volume, good separation was obtained without any degradation of peak shape. The detection limits (with SN ratio = 3.3) using the 50 µL injection were 1 ng/L (1 ppt) for aflatoxin B_1, and 2 ng/L (2 ppt) for aflatoxin G_1.

■Analytical Conditions

Column	: Shim-pack XR-ODS II (100 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile Phase	: Water / Methanol / Acetonitrile = 6/3/1 (v/v/v)
Flowrate	: 1.0 mL/min
Column Temp.	: 50 °C
Injection Volume	: 8 µL
Detection	: RF-20Axs Ex. at 365 nm, Em. at 450 nm
RF Cell	: Conventional cell
Cell Temp.	: 25 °C

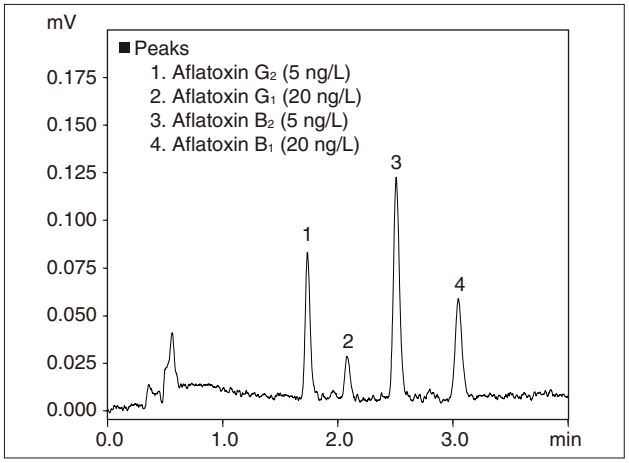


Fig. 6.2.1 Chromatogram of a Standard Mixture of Aflatoxins (8 µL injected)

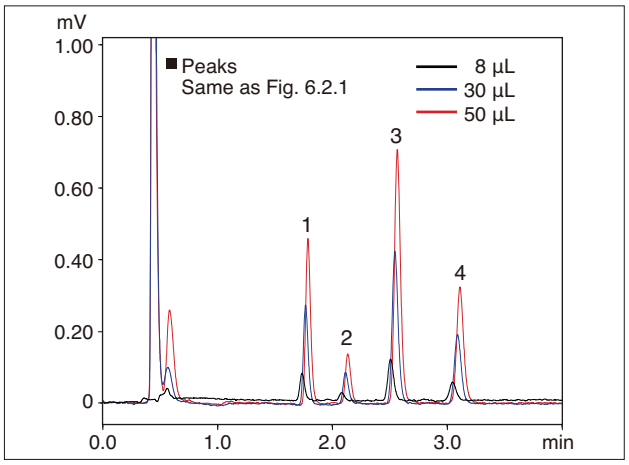


Fig. 6.2.2 Chromatograms of a Standard Mixture of Aflatoxins-Comparison of Injection Volume

6.2 High Speed Analysis of Aflatoxins in Food (2) - LC

■Effect of Cell Temperature Control

It is generally known that fluorescence intensity is easily affected by the surrounding temperature, specifically that it is diminished or "quenched" at higher temperatures. Fig. 6.2.3 shows the relationship between the RF-20Axs cell temperature and the peak height ratio (based on a peak height of 1 at 20 °C). From these results, it is clear that aflatoxins B_1 and B_2 are particularly affected by room temperature fluctuation. Because the RF-20Axs is equipped with a cell temperature control feature as standard, high accuracy analysis is possible without the adverse effect of room temperature fluctuation, even in the high-sensitivity analysis of aflatoxins.

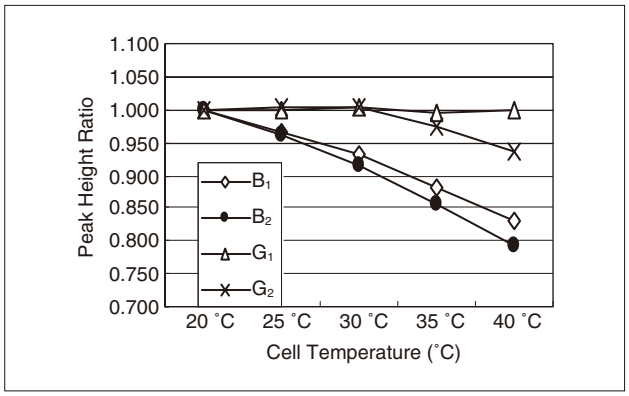


Fig. 6.2.3 Relationship between Cell Temperature and Peak Height Ratio

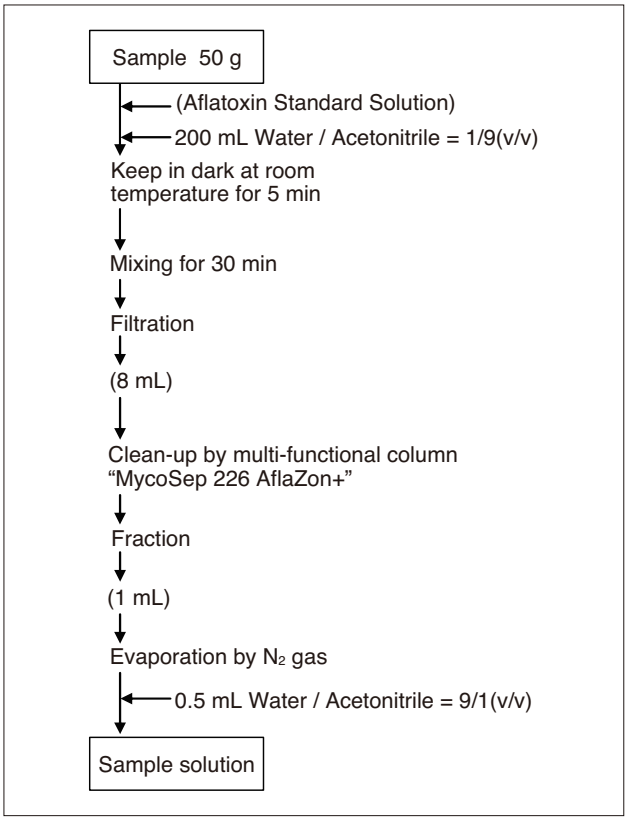


Fig. 6.2.4 Sample Preparation

■Analysis of Food Sample

We conducted sample preparation of commercially available wheat flour according to the procedure shown in Fig. 6.2.4. In addition, the same preparation was also conducted for the same flour, but spiked with a standard mixture of 4 aflatoxins (B_1 and G_1 at 0.8 µg/kg, and B_2 and G_2 at 0.2 µg/kg). These samples were analyzed using the same analytical conditions as shown on the previous page. Fig. 6.2.5 shows the respective chromatograms.

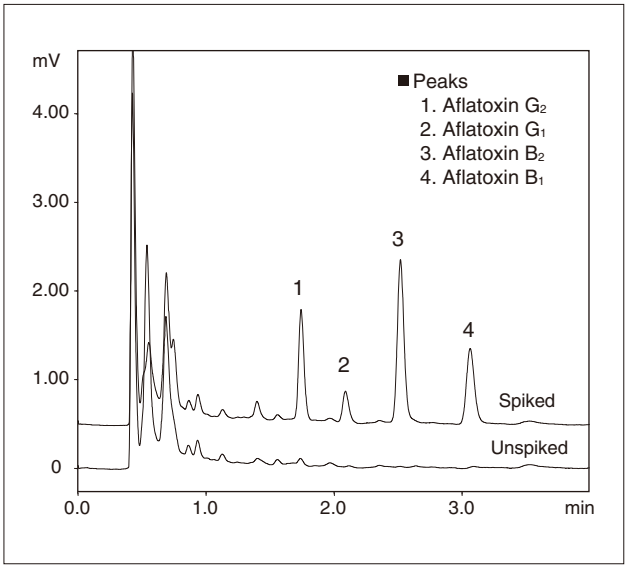


Fig. 6.2.5 Chromatograms of Wheat Flour Samples (8 µL injected)
(Upper: Spiked, Lower: Unspiked)

6.3 Analysis of Aflatoxin B_1, B_2, G_1 and G_2 at High Sensitivity (1) - LC

■Explanation

Aflatoxins are toxins produced by the fungus Aspergillus. In addition to being acutely toxic, they are also known to be carcinogenic, so their inspection in foods is necessary[1]. Up to now, aflatoxin B_1 is the only aflatoxin that has been regulated in Japan. However, beginning October 2011, the restriction extends to the total of aflatoxin B_1, B_2, G_1 and G_2.[2].

Here, we introduce two cases of analysis of these 4 aflatoxins (B_1, B_2, G_1, G_2), using the Prominence RF-20Axs high-sensitivity fluorescence detector, first by fluorescence detection using trifluoroacetic acid derivatization, and then direct detection without TFA derivatization.

■Derivatization of Aflatoxins with Trifluoroacetic Acid

Typically, aflatoxins B_1 and G_1 are converted to the hydroxyl-derivative aflatoxins B_{2a} and G_{2a} using trifluoroacetic acid (TFA) to increase their fluorescence intensity in HPLC analysis. Fig. 6.3.1 shows the structures of the 4 aflatoxins and those of the B_{2a} and G_{2a} TFA derivatives.When analyzing aflatoxins in food, TFA derivatization is done for both the standard solution and the sample solution.

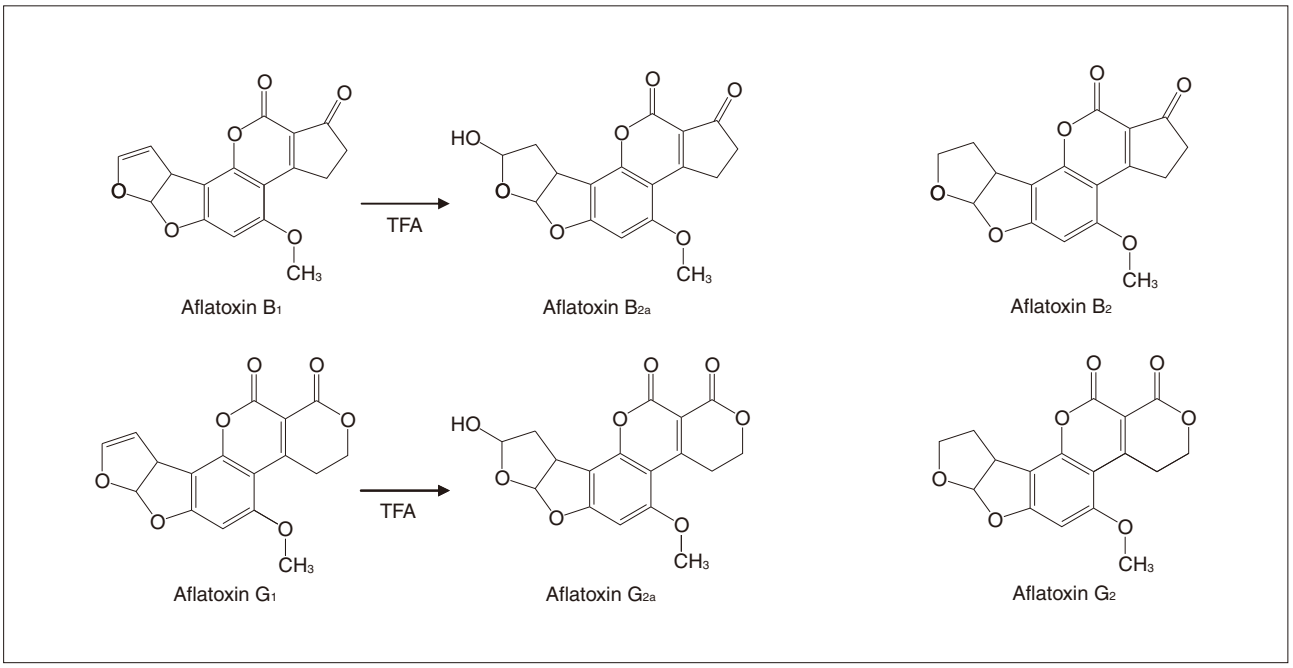


Fig. 6.3.1 Structures of Aflatoxins B_1, B_2, G_1, G_2, and Trifluoroacetic Acid-Derivatized Forms (B_{2a}, G_{2a})

6.3 Analysis of Aflatoxin B_1, B_2, G_1 and G_2 at High Sensitivity (2) - LC

■Analysis of Standard Solution After Derivatization with Trifluoroacetic Acid

Fig. 6.3.3 shows chromatograms of standard solutions of the 4 aflatoxins (B_1, B_2, G_1, G_2) obtained following TFA derivatization (Fig. 6.3.2) with 20 µL injections. Regarding the standard solution B_{2a} peak in the chromatogram on the right in Fig. 6.3.3, a peak area repeatability (n=6) of 1.2 %RSD was obtained, and the detection limit (S/N ratio=3.3, 20 µL injection) was calculated to be 0.4 ng/L (8 fg). Ultra-trace levels of aflatoxins can accurately be detected with high sensitivity using the RF-20Axs.

■Analytical Conditions

Column	: Shim-pack FC-ODS (150 mm L. × 4.6 mm I.D., 3 µm)
Mobile Phase	: Water / Methanol / Acetonitrile = 6/3/1 (v/v/v)
Flowrate	: 0.8 mL/min
Column Temp.	: 40 °C
Detection	: RF-20Axs, Ex at 365 nm, Em at 450 nm
RF Cell	: Conventional cell
Cell Temp.	: 25 °C
Injection Volume	: 20 µL

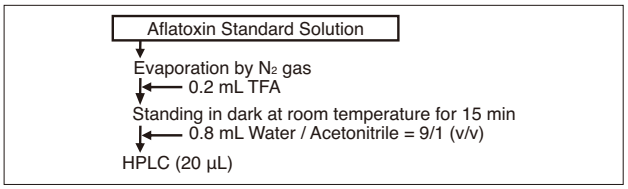


Fig. 6.3.2 Derivatization with TFA

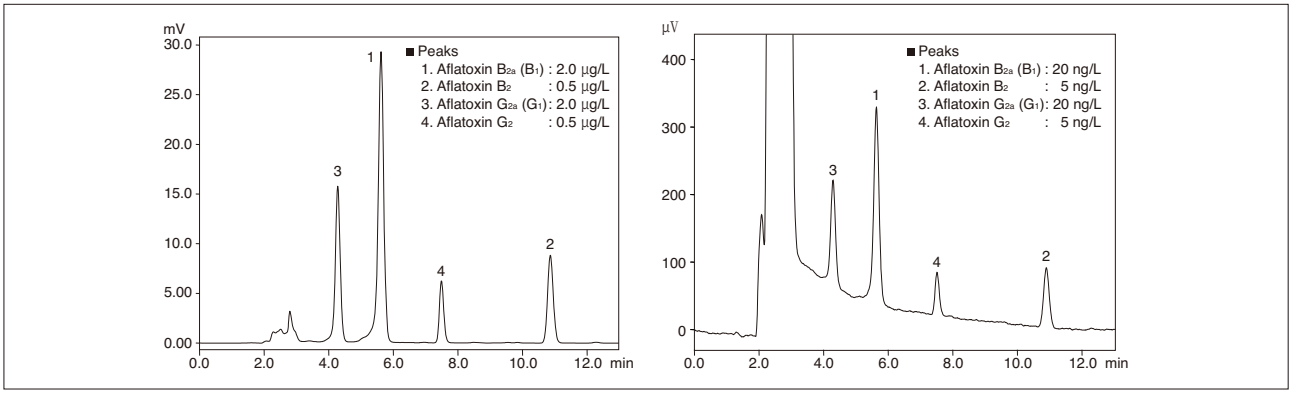


Fig. 6.3.3 Chromatograms of Aflatoxin Standard Solutions After Derivatization with Trifluoroacetic Acid (20 µL injected)
(Left) B_1 and G_1: 2.0 µg/L, B_2 and G_2: 0.5 µg/L, (Right) B_1 and G_1: 20 ng/L, B_2 and G_2: 5 ng/L

■Analysis of Standard Solution by Direct Detection

Fig. 6.3.4 shows chromatograms of standard solutions of the 4 aflatoxins (B_1, B_2, G_1, G_2) obtained with 20 µL injections, without TFA derivatization. The analytical conditions were the same as those shown on this page. Regarding the B_1 peak in the chromatogram on the right in Fig. 6.3.4, a peak area repeatability (n=6) of 2.7 %RSD was obtained, and the detection limit (S/N ratio=3.3, 20 µL injection) was calculated to be 3 ng/L (60 fg). This demonstrated that testing for aflatoxin B_1 and G_1 can be done with sufficient sensitivity using direct detection with the RF-20Axs, without TFA derivatization.

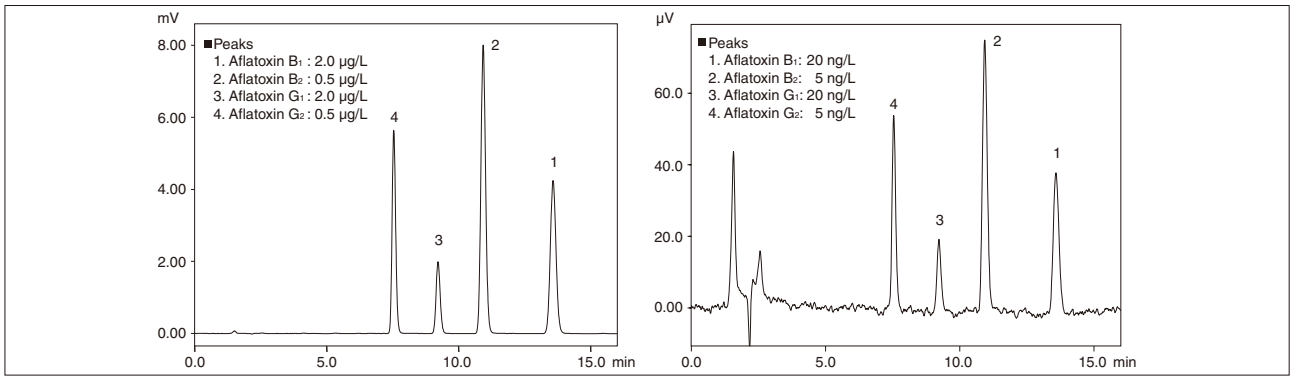


Fig. 6.3.4 Chromatograms of Aflatoxin Standard Solutions by Direct Detection (20 µL injected)
(Left) B_1 and G_1: 2.0 µg/L, B_2 and G_2: 0.5 µg/L, (Right) B_1 and G_1: 20 ng/L, B_2 and G_2: 5 ng/L

6.3 Analysis of Aflatoxin B_1, B_2, G_1 and G_2 at High Sensitivity (3) - LC

■Analysis of Food

For a food application example, aflatoxin standard solution was added to commercially available corn flour so that the respective aflatoxin concentrations in the sample became 0.8 µg/kg for B_1 and G_1, and 0.2 µg/kg for B_2 and G_2. The pseudo contaminated sample was then analyzed. Fig. 6.3.5 shows the sample preparation procedure. The contaminants were removed using a multifunctional column.

Analysis was conducted using both TFA derivatization and direct detection without derivatization. Fig. 6.3.6 shows the chromatograms obtained from analysis of commercially available corn flour that was spiked and unspiked (blank) with the aflatoxin standard solution, in this case using TFA derivatization. Fig. 6.3.7 shows the chromatograms obtained using direct detection analysis without conducting derivatization.

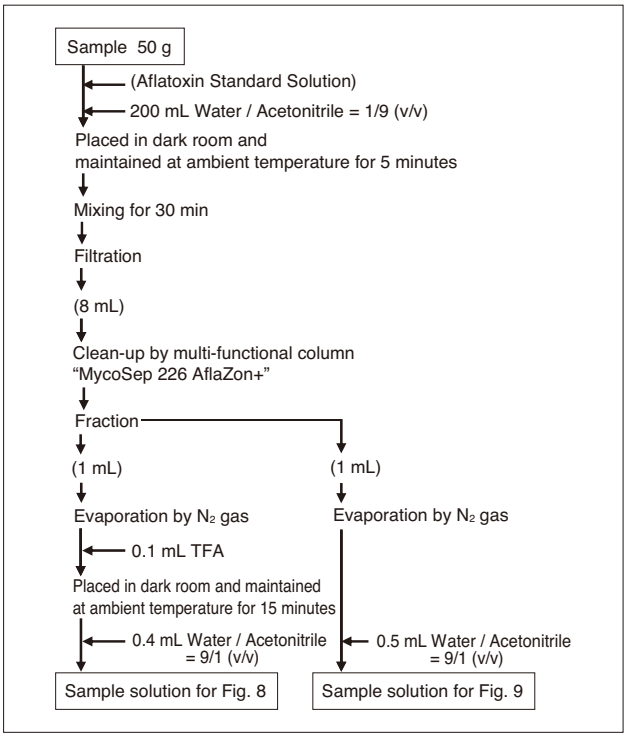


Fig. 6.3.5 Sample Preparation

■Analytical Conditions

Mobile Phase : A: Water / Methanol / Acetonitrile = 6/3/1 (v/v/v)
B: Acetonitrile
Gradient Elution Method

Time Program : B 0 % (0.00-15.00 min)→90 %
(16.00-23.00 min) → 0 % (24.00-34.00 min)

(The other analytical conditions are the same as those on the previous page)

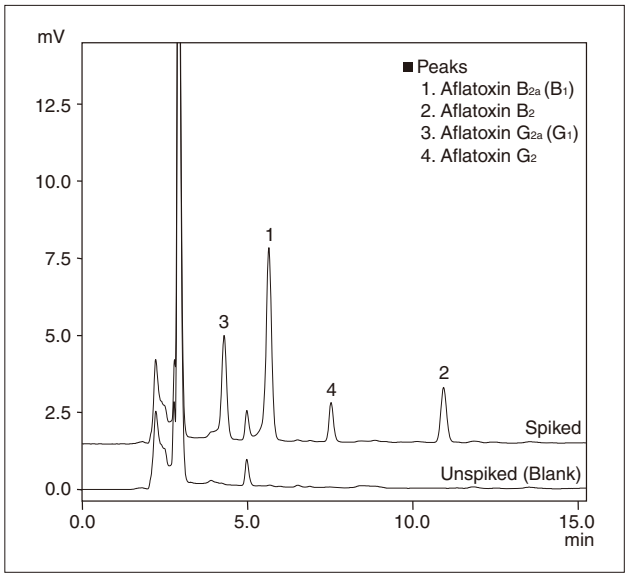


Fig. 6.3.6 Chromatograms of Corn Flour Using Derivatization with TFA (20 µL injected)
(Upper) Spiked with Aflatoxin Standard, (Lower) Unspiked

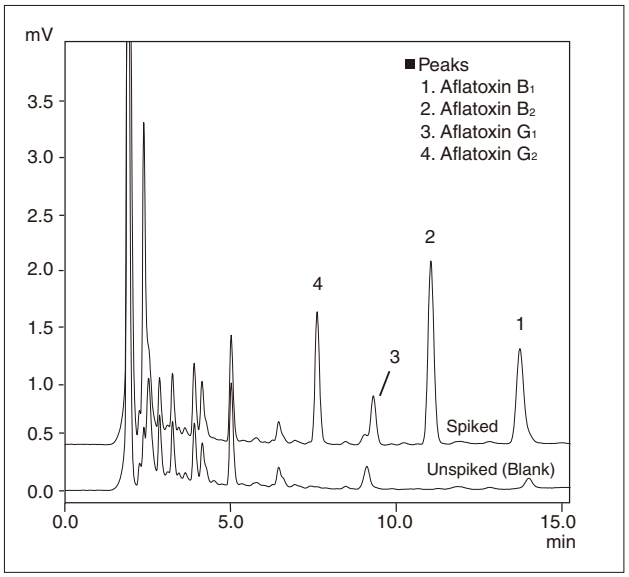


Fig. 6.3.7 Chromatograms of Corn Flour by Direct Detection (20 µL injected)
(Upper) Spiked with Aflatoxin Standard, (Lower) Unspiked

[References]

1) Handling of Foods Containing Mycotoxins (Aflatoxins) (Notification No. 128, issued on March 16, 1971 by the Environmental Health Bureau, Ministry of Health, Labour and Welfare; revised on March 26, 2002 by the Food Inspection Division [No. 0326001], Japan)
2) Handling of Foods Containing Aflatoxins (Notification 0331, No. 5, issued on March 31, 2011 by the Food Safety Division, Ministry of Health, Labour and Welfare, Japan)

6.4 High Speed Analysis of Nivalenol and Deoxynivalenol (1) - LC

■Explanation

Nivalenol and deoxynivalenol are trichothecene mycotoxins which are produced by Fusarium fungi, and are known to be associated with digestive system disorders and toxicity to the immune system if they enter the food chain through contaminated cereal crops. Here we introduce an example of high-speed analysis of nivalenol and deoxynivalenol* using the ultra-fast LC system Prominence UFLC with the high-speed, high resolution, high-performance Phenomenex Synergi Hydro-RP column.

* The standard mixture of nivalenol and deoxynivalenol, and the flour extract solution were provided by the Japan Grain Inspection Association.

■Analysis of Standard Solution

Fig. 6.4.1 shows the structures of nivalenol and deoxynivalenol. Because these are highly polar compounds, we used the Phenomenex Synergi Hydro-RP (2.5 µm particle diameter) high-speed, high resolution ODS column, in which the bonded phase is endcapped with a polar group to provide enhanced retention, while also providing increased longevity with highly aqueous mobile phases. Fig. 6.4.2 shows the analysis results of the standard mixture of nivalenol and deoxynivalenol (prepared by dissolving each in acetonitrile at 1.0 mg/L, and then diluting with mobile phase), using a 20 µL injection. The peak in the vicinity of 2 minutes on the chromatogram derives from dissolved oxygen in the sample solution[1], but does not interfere with the complete separation of nivalenol and deoxynivalenol when using the Phenomenex Synergi Hydro-RP column.

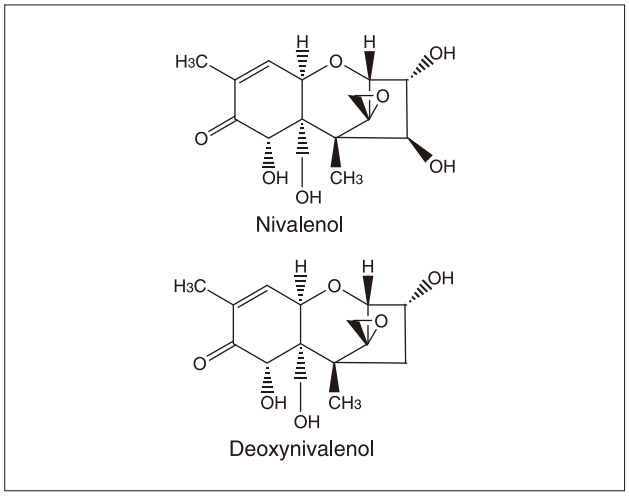


Fig. 6.4.1 Structures of Nivalenol and Deoxynivalenol

■Analytical Conditions

Column	: Phenomenex Synergi 2.5 µm Hydro-RP 100 Å (100 mm L. × 3.0 mm I.D., 2.5 µm)
Mobile Phase	: A: 10 mmol/L (Sodium) Phosphate Buffer (pH 2.6) B: Acetonitrile C: Methanol A / B / C = 18/1/1 (v/v/v)
Flowrate	: 0.9 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 20 µL
Detection	: SPD-20AV at 220 nm
UV Cell	: Semi-micro Cell

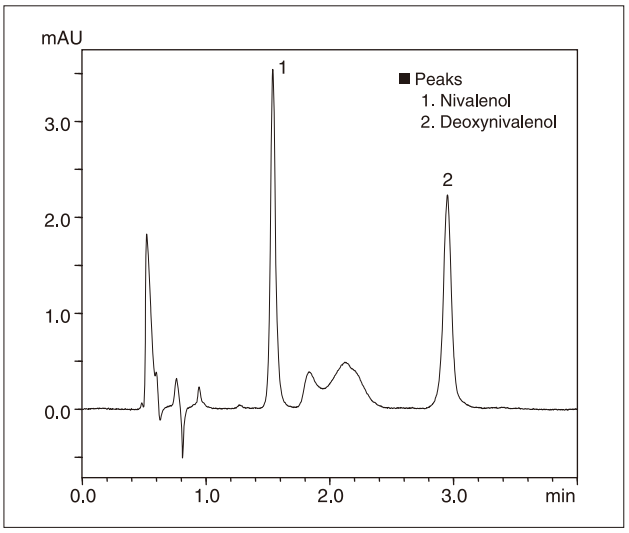


Fig. 6.4.2 Chromatogram of a Standard Mixture of Nivalenol and Deoxynivalenol (1.0 mg/L each, 20 µL injected)

6.4 High Speed Analysis of Nivalenol and Deoxynivalenol (2) - LC

■Analysis of Flour

Fig. 6.4.3 shows the results of analysis of a flour extract solution contaminated with trichothecene mycotoxins. Here, the gradient elution method was used for the purpose of conducting column washing. The flour sample preparation procedure is shown in Fig. 6.4.4. In Japan, the provisional standard criteria value for deoxynivalenol content in flour is set to 1.1 ppm[2], but the concentration of deoxynivalenol in this sample was 0.60 ppm.

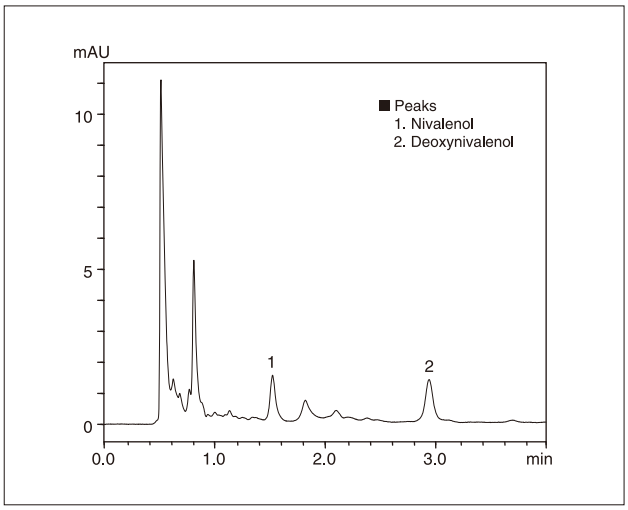


Fig. 6.4.3 Chromatogram of Flour Extract

■Analytical Conditions

Column	: Phenomenex Synergi 2.5 µm Hydro-RP 100 Å (100 mm L. × 3.0 mm I.D., 2.5 µm)
Mobile Phase	: A) a / b / c = 18/1/1 (v/v/v)
	: B) a / b / c = 5/4/1 (v/v/v)
	a: 10 mmol/L (Sodium) Phosphate Buffer (pH 2.6)
	b: Acetonitrile
	c: Methanol
	Gradient Elution Method
Time Program	: B 0 % (0-4 min) → 100 % (4.01-5.5 min) → 0 % (5.51-12 min)
Flowrate	: 0.9 mL/min
Column Temp.	: 40 ˚C
Injection Vol.	: 20 µL
Detection	: SPD-20AV at 220 nm
UV Cell	: Semi-micro cell

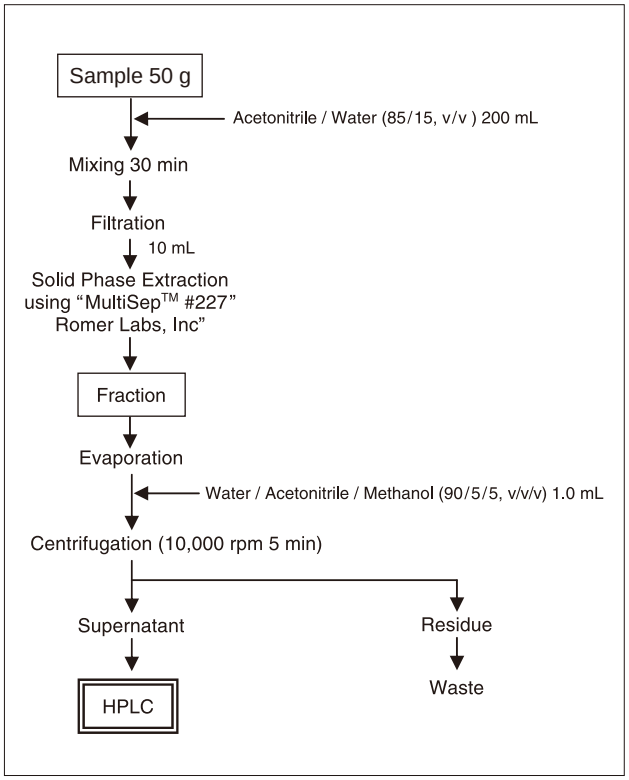


Fig. 6.4.4 Sample Preparation

[References]
1) "Test Method for Deoxynivalenol" (Food Safety Notification No. 071702, Ministry of Health, Labour and Welfare, July 17, 2003, Japan)
2) "Provisional Standard Criteria Value for Deoxynivalenol" (Food Safety Notification No. 0521001, Ministry of Health, Labour and Welfare, May 21, 2002, Japan)

6.5 Analysis of Arsenic in Foods (1) - EDX

■Explanation

X-ray fluorescence spectrometers enable quickly, easily, and non-destructively analyzing solids, powders, liquids, and other samples. In particular, they are useful for detecting and quantitatively analyzing arsenic (As), cadmium (Cd), and potassium (K), and other elements in toxic substances in foods. The following is an example of analyzing arsenic levels of sodium arsenite mixed in oolong tea, juice, and curry. EDX-700/800 models include five types of primary filters as standard accessories, which are required for trace analysis of substances such as arsenic. An example of trace analysis is also described below.

■Samples

Samples were prepared by mixing sodium arsenite ($NaAsO_2$) with commercially marketed oolong tea, juice, and curry (in retort packaging) to an arsenic concentration of about 0.4 %.

About 6 mL of untreated samples were placed in liquid containers with 6 µm thick PET film adhered to the bottom.

■Analytical Conditions

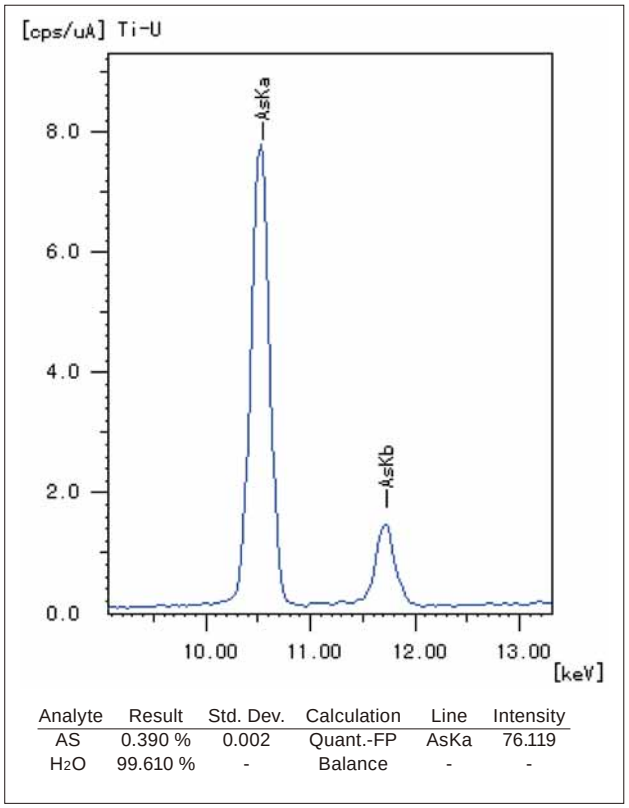
Instrument	: EDX-700
X-Ray Tube	: Rh target
Filter	: Ni or without
Voltage - Current	: 50 kV – 15 µA (auto)
Atmosphere	: Air
Measurement Diameter	: 10 mm
Measurement Time	: 40 sec
Dead Time	: 25 %

■Analysis Results

Qualitative analysis results for oolong tea, juice, and curry are shown in Fig. 6.5.1, Fig. 6.5.2, and Fig. 6.5.3. Quantitative analysis values are indicated in Table 6.5.1. Equations were balanced assuming H_2O (water) as the primary ingredient and ignoring all non-arsenic elements.

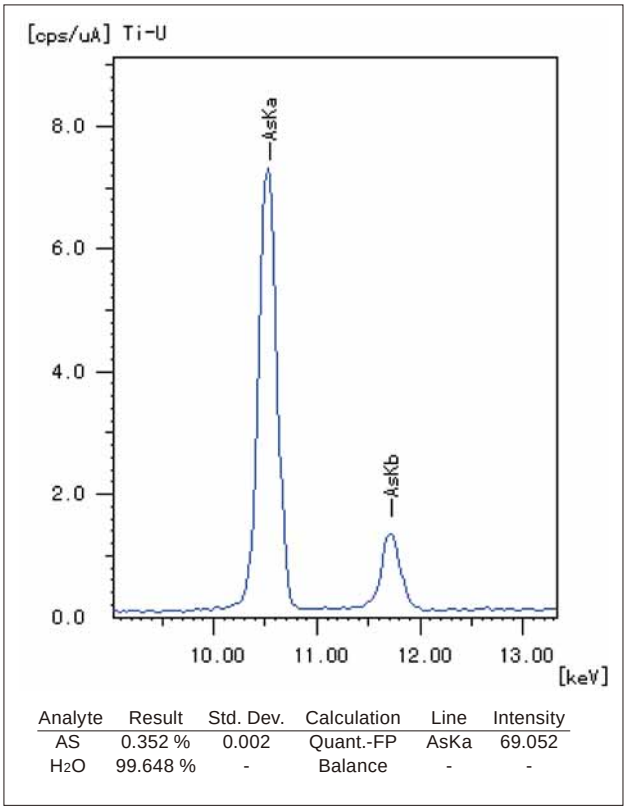
Table 6.5.1 Quantitative Value of As in Foods

Element	Oolong Tea	Juice	Curry
As	0.39 %	0.35 %	0.33 %
H_2O	99.61 %	99.65 %	99.67 %



Analyte	Result	Std. Dev.	Calculation	Line	Intensity
AS	0.390 %	0.002	Quant.-FP	AsKa	76.119
H_2O	99.610 %	-	Balance	-	-

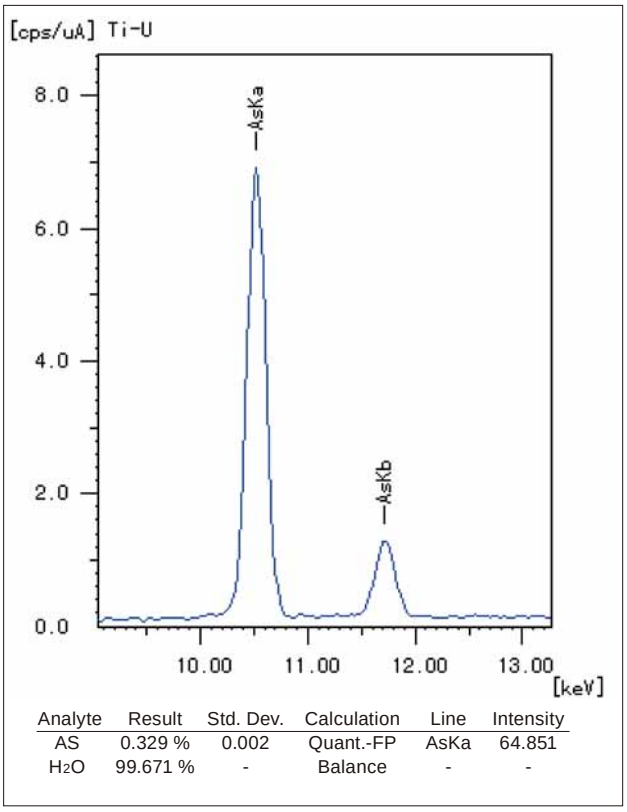
Fig. 6.5.1 Qualitative and Quantitative Analysis of Oolong Tea



Analyte	Result	Std. Dev.	Calculation	Line	Intensity
AS	0.352 %	0.002	Quant.-FP	AsKa	69.052
H_2O	99.648 %	-	Balance	-	-

Fig. 6.5.2 Qualitative and Quantitative Analysis of Juice

6.5 Analysis of Arsenic in Foods (2) - EDX



Analyte	Result	Std. Dev.	Calculation	Line	Intensity
AS	0.329 %	0.002	Quant.-FP	AsKa	64.851
H_2O	99.671 %	-	Balance	-	-

Fig. 6.5.3 Qualitative and Quantitative Analysis of Curry

■EDXRF Analysis of Trace Arsenic Using Primary Filter

Trace analysis requires using a primary filter. Using a primary filter enables reducing the scattering of primary X-rays from the X-ray tube to obtain measurements with good S/N ratios. Consequently, heavy metals such as arsenic can be detected down to a few ppm. The following is an example of analyzing trace arsenic in an aqueous solution using a nickel primary filter. The nickel filter is also required for trace analysis of $_{29}$Cu, $_{30}$Zn ... $_{42}$Mo, $_{73}$Ta, $_{74}$W ... and $_{92}$U as well.

■Samples

A 10 ppm aqueous solution was used, containing a standard 1000 ppm solution of arsenic for atomic absorption spectrometry, diluted by 100 times.

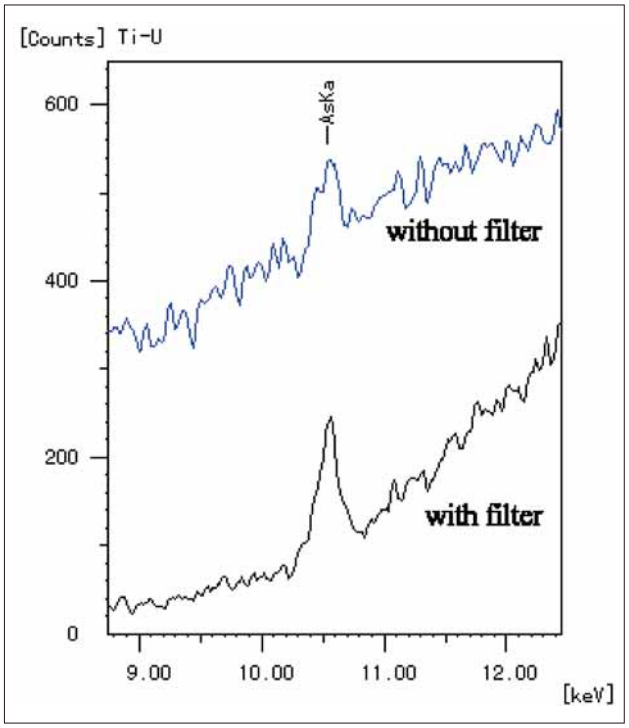


Fig. 6.5.4 Qualitative Analysis With and Without Ni Filter

Table 6.5.2 Detection Limits* of As in Aqueous Solution

With Ni Filter	Without Ni Filter
0.9 ppm	2.0 ppm

*Detection limits calculated as follows:

$$L.L.D. = 3\frac{C}{I\,net}\sqrt{\frac{I_{back}}{T}}$$

C : Standard value
$I\,net$: Net intensity
$I\,back$: Background intensity
T : Measurement time

■Analysis Results

Qualitative analysis results from the 10 ppm aqueous arsenic solution with and without using a nickel filter are shown overlaid in Fig. 6.5.4.
Based on these results, detection limits were calculated for with and without using a nickel filter, as shown in Table 6.5.2.

7.1 High Sensitivity Analysis of 2,4,6-Trichloroanisole in Wine (1) - GC/MS/MS

Introduction

2,4,6-trichloroanisole (TCA) emitted from wine corks can taint wine and cause an objectionable odor. Due to the low threshold value for sensing the odor, highly sensitive measurements are required for monitoring. Conventionally, TCA was measured using methods such as purge and trap, which is very effective in concentrating samples, or thermal desorption. The HS-20 headspace sampler includes a trap function that is able to concentrate headspace gases. Here we introduce an example of high-sensitivity measurement of TCA in wine using an HS-trap GC/MS system. The structure of TCA is illustrated in Fig. 7.1.1 and the mass spectrum is shown in Fig. 7.1.2.

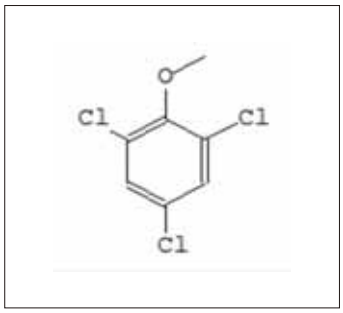


Fig. 7.1.1 Structure of TCA

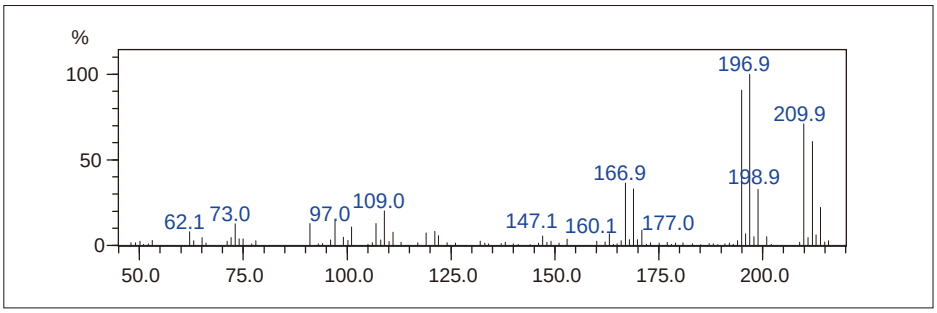


Fig. 7.1.2 Mass Spectrum of TCA

Analytical Conditions

HS-20

Mode	: Trap
Equilibrating Time	: 30 min
Oven Temp.	: 60 °C
Sample Line Temp.	: 260 °C
Transfer Line Temp.	: 260 °C
Trap Equilibrating Temp.	: 80.0 °C
Trap Cooling Temp.	: 80.0 °C
Trap Desorbing Temp.	: 280.0 °C
Vial Pressurizing Time	: 2.0 min
Pressure Equilibrating Time	: 0.1 min
Load Time	: 0.1 min
Load Equilibrating Time	: 0.1 min
Dry Purge	: 5 min
Injection Time	: 20 min
Needle Flush	: 20 min
Injection Cycle	: 3 cycles
Cycle Time	: 50 min
Sample Loading Volume	: 5 mL

GCMS-QP2010 Ultra

GC Unit	
Column	: Rxi-5ms 0.32 mm I.D. × 60 mL., df = 1.0 µm
Column Temp.	: 50 °C (1 min) - 10 °C/min - 300 °C (5 min)
Carrier Gas Control	: Constant pressure
Carrier Gas Pressure	: 180 kPa
Injection Mode	: Splitless
Sampling Time	: 3 min
Additional Flow	
APC1	: 100 kPa
APC3	: 50 kPa
MS Unit	
Interface Temp.	: 280.0 °C
Ion Source Temp.	: 230.0 °C
Solvent Elution Time	: 14 min
Measurement Start Time	: 15 min
Measurement End Time	: 20 min
Measurement Mode	: SIM
Selected Ions (*m/z*)	: 211.9, 209.9, 196.9, 194.9
Event Time	: 0.2 sec

7.1 High Sensitivity Analysis of 2,4,6-Trichloroanisole in Wine (2) - GC/MS/MS

■Sensitivity

A wine sample spiked with the equivalent of 1 ng/L TCA was measured by SIM using the HS-trap method (Fig. 7.1.3). The results show how the system was able to analyze low concentrations of TCA with high sensitivity. A wine sample spiked with the equivalent of 100 ng/L TCA was measured by SIM using the headspace-GC/MS method and HS-trap method, as shown in Fig. 7.1.4 and Fig. 7.1.5. A comparison of both shows that the HS-trap method provided about 10 times higher sensitivity.

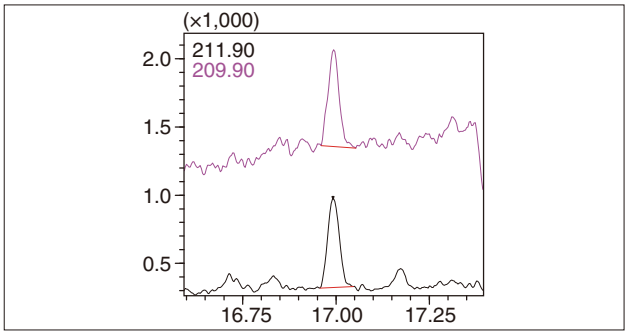


Fig. 7.1.3 SIM Chromatogram of TCA in Wine Measured Using HS-Trap (Wine spiked with 1 ng/L TCA)

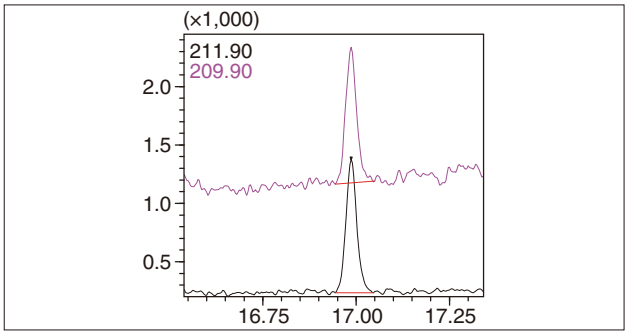


Fig. 7.1.4 SIM Chromatogram of TCA in Wine Measured Using Conventional Headspace GC/MS (Wine spiked with 100 ng/L TCA)

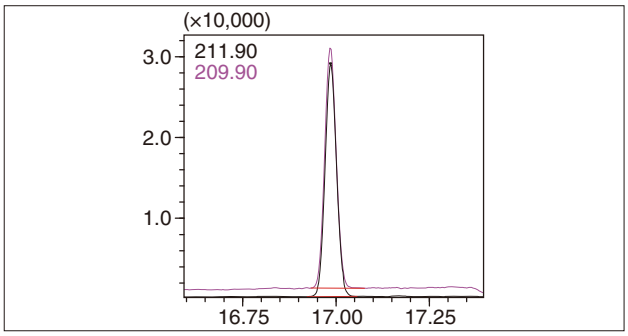


Fig. 7.1.5 SIM Chromatogram of TCA in Wine Measured Using HS-Trap (Wine spiked with 100 ng/L TCA)

■Linearity and Repeatability

Linearity was confirmed by adding specific concentrations of trichloroanisole to wine (from 1 to 100 ng/L, as shown in Fig. 7.1.6). The results showed good linearity. 3 ng/L of trichloroanisole was added to wine to test the repeatability (n=5) of peak area (Table 7.1.1). Results showed good repeatability, with a CV value not exceeding 5 %.

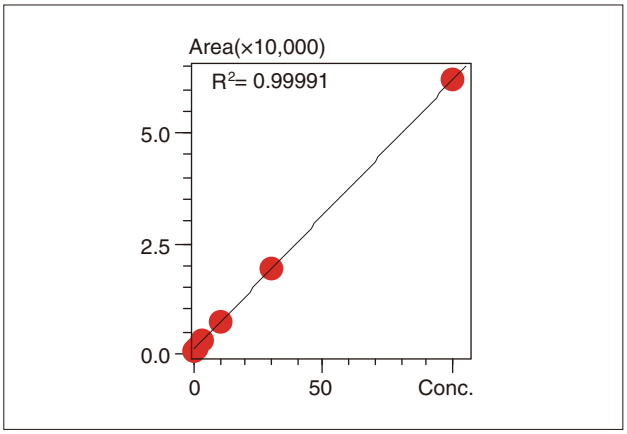


Fig. 7.1.6 Linearity of TCA (Wine spiked with 1-100 ng/L TCA)

Table 7.1.1 Area Repeatability of TCA (n=5, wine spiked with 3 ng/L TCA)

AREA	1	2	3	4	5	Average	%RSD
TCA *m/z* 211.9	3,103	3,051	2,925	3,020	2,742	2,968	4.79 %

■Conclusion

We presented here an example of high-sensitivity measurement of trichloroanisole in wine using an HS-trap GC/MS system. The results showed that the system was easily able to measure even a few nanograms per liter. This also confirmed that an HS-trap-GC/MS system using the HS-20 headspace sampler is effective in monitoring trichloroanisole in wine.

7.2 Rapid Determination of Melamine in Food (1) - LC

■Explanation

Melamine is used as the main raw material of melamine resin, a thermosetting plastic, but recently it is receiving considerable attention due to numerous incidents of food contamination with this substance. Analysis of melamine in food can be performed using HPLC, LC/MS(/MS), and GC/MS, etc., depending on the objective, but among these, HPLC allows for easy, multi-specimen screening analysis. Here we introduce a rapid analytical technique for determining melamine in food using the ultra-fast LC, Prominence UFLC together with a simple sample preparation procedure.

■Analysis of Standard Solution

Melamine (Fig. 7.2.1) is a high-polarity substance with a triazine ring, but since it is not sufficiently retained using a typical reversed-phase mode, we used the reversed-phase ion pairing mode for this analysis. Detection was conducted with a photodiode array detector, and the Shim-pack XR-ODS high-performance, high-resolution column (particle size 2.2 µm) was used for the analytical column. Fig. 7.2.2 shows the UV spectrum of melamine. Quantitation was conducted at the maximum absorption wavelength of 235 nm. Fig. 7.2.3 shows the chromatogram of a melamine standard solution (1000 µg/L).

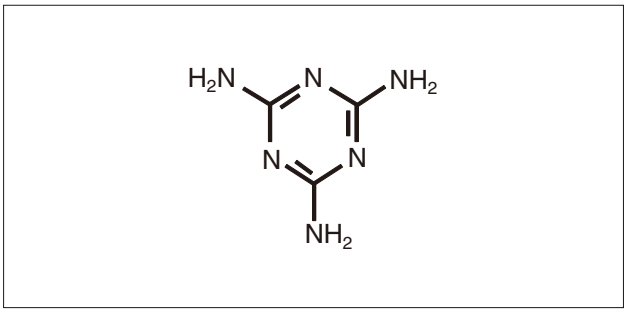


Fig. 7.2.1 Structure of Melamine

■Analytical Conditions

Column	: Shim-pack XR-ODS (75 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile Phase	: A: 10 mmol/L (Sodium) Phosphate Buffer (pH 2.6) containing 10 mmol/L Sodium 1-octanesulfonate B: Acetonitrile A / B = 92/8 (v/v)
Flowrate	: 1.2 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 10 µL
Detection	: SPD-M20A at 235 nm
Flow Cell	: Conventional cell

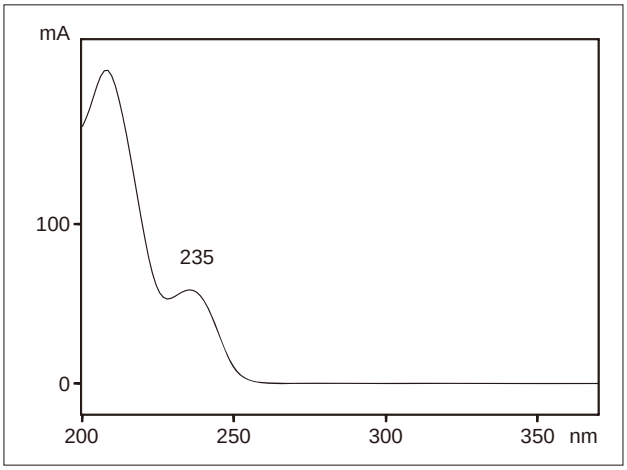


Fig. 7.2.2 UV Spectrum of Melamine

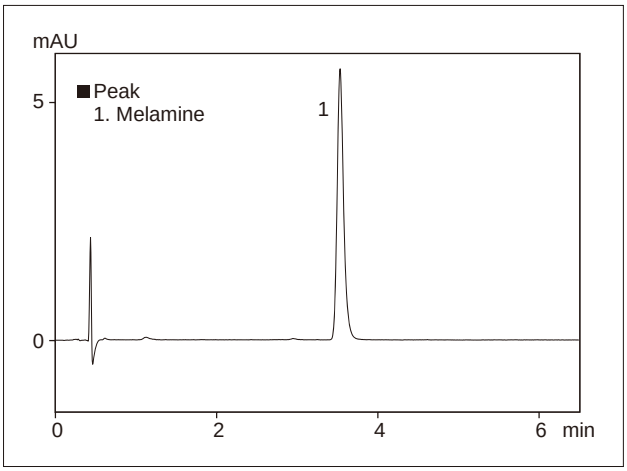


Fig. 7.2.3 Chromatogram of Melamine Standard Solution (1000 µg/L)

7.2 Rapid Determination of Melamine in Food (2) - LC

■Linearity and Sensitivity

The linearity of melamine detection using standard solution concentrations of 10 µg/L to 100 µg/L is shown in Fig. 7.2.4. Excellent linearity is obtained with a correlation coefficient (R^2) greater than 0.9999. The quantitation limit (S/N=10) and detection limit (S/N=3.3) with injection of 10 µL were 16 µg/L and 5 µg/L, respectively.

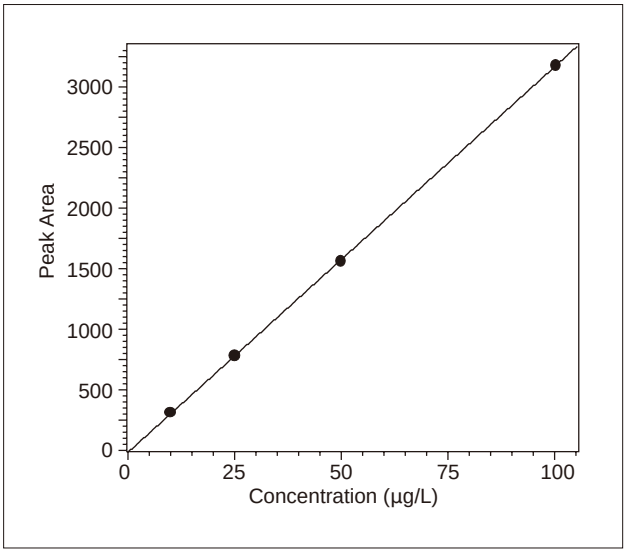


Fig. 7.2.4 Linearity (10 µg/L to 100 µg/L)

■Sample Preparation

A simple method based on methanol deproteinization was examined as a food preparation method for rapid screening analysis. Fig. 7.2.5 shows the sample preparation procedure for analysis of milk and wheat flour.

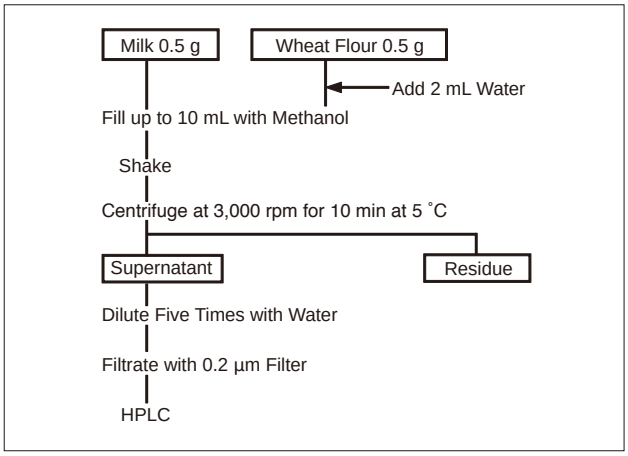


Fig. 7.2.5 Sample Preparation

■Analysis of Melamine in Food

Melamine equivalent to 2 mg/kg was added to each portion of commercially available milk and wheat flour, and sample preparation was conducted according to the procedure of Fig. 7.2.5 (final prepared liquid containing 20 µg/L concentration of melamine in each). The analytical results are shown in Fig. 7.2.6 and Fig. 7.2.7.* The recovery of added melamine in this analysis was 97 % for the milk, and 73 % for the wheat flour.

* It is recommended to rinse the column (for example, mobile phase: A / B = 1/1) after completion of each analysis.

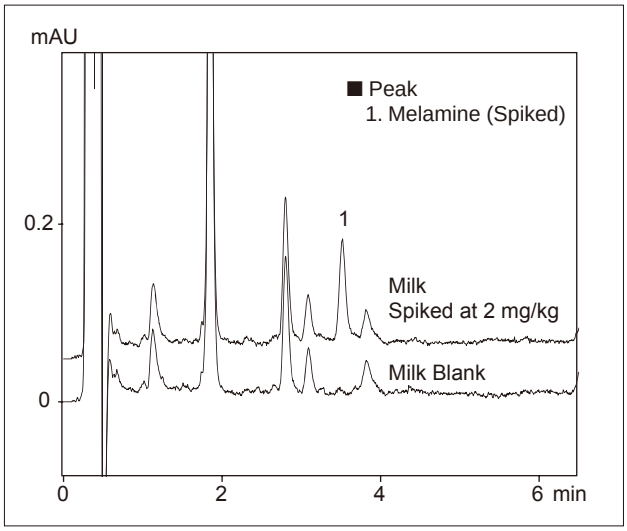


Fig. 7.2.6 Chromatograms of Milk

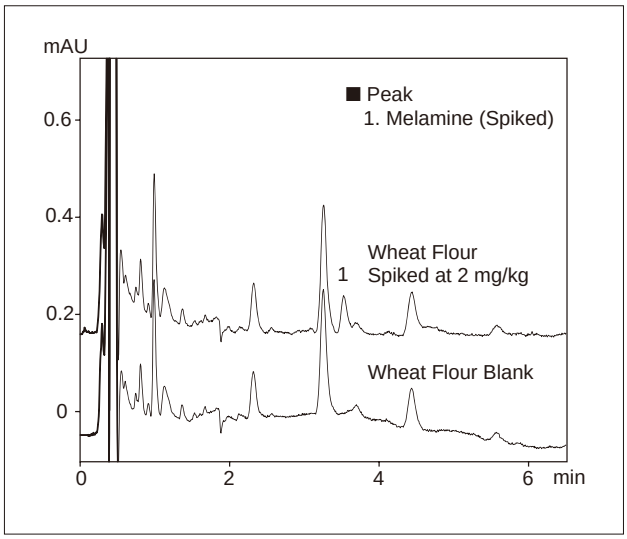


Fig. 7.2.7 Chromatograms of Wheat Flour

7.3 Analysis of Contaminant Adhering to Frozen Pizza (1) - FTIR/EDX/EPMA

■Explanation

The steady stream of consumer complaints related to foods reflects continuing high concern for food safety. To address these concerns and specific contamination-related complaints, it is important to quickly and accurately report the analysis results and clearly elucidate the contamination pathway. Here, using a Fourier transform infrared spectrometer (FTIR) and an energy dispersive X-ray fluorescence spectrometer (EDX), in addition to an electron probe micro analyzer (EPMA), we present the results of analysis of a contaminant adhering to the surface of frozen pizza.

■Photograph of Contaminant Adhering to Surface Frozen Pizza

The photograph in Fig. 7.3.1 shows the site of contamination on the frozen pizza. It was discovered when the package of this commercially available frozen pizza was opened. The foreign substance was subjected to complex analysis using a Fourier transform infrared spectrometer (FTIR), an energy dispersive X-ray fluorescence spectrometer (EDX), and an electron probe micro analyzer (EPMA).

Fig. 7.3.1 Photograph of Contaminant Adhering to Frozen Pizza

■Analysis by FTIR

Some of the foreign substance was scraped off the frozen pizza, and after rolling it onto the diamond cell, the infrared spectrum was measured by transmission infrared microscopy. Measurements were conducted at multiple sites on the contaminant. Fig. 7.3.2 shows a photograph indicating the measurement locations, and Fig. 7.3.3 shows the overlaid infrared spectra that were obtained at the respective sites. Measurements were conducted using 30 × 30 μm focal regions. The analytical conditions used are shown in Table 7.3.1.

Table 7.3.1 Instruments and Analytical Conditions

Instruments	: IRPrestige-21, AIM-8800
Resolution	: 4 cm^{-1}
Accumulation	: 45
Apodization	: Happ-Genzel
Detector	: MCT

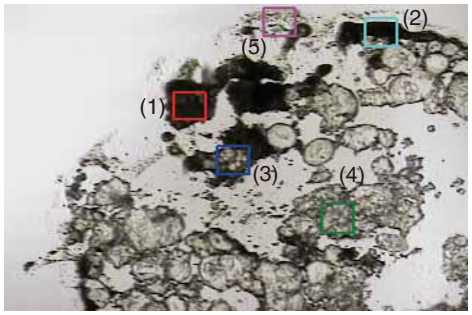


Fig. 7.3.2 Microscope Photograph of Measurement Sites

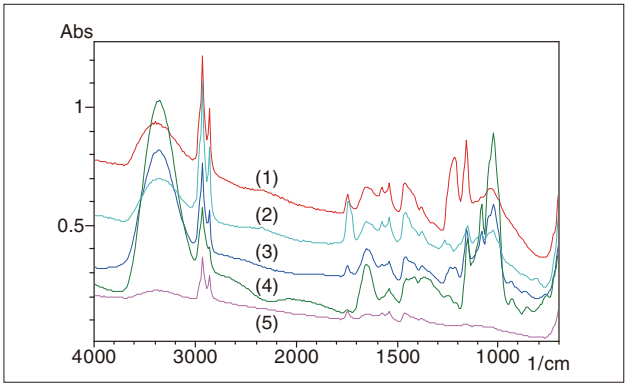


Fig. 7.3.3 Infrared Spectra of Contaminants

It is evident from Fig. 7.3.3 that the infrared spectra appear differently depending on the location. Among the spectra of Fig. 7.3.3, searches were conducted using the spectra (1), (4) and (5). The results are shown in Fig. 7.3.4 - Fig. 7.3.6.

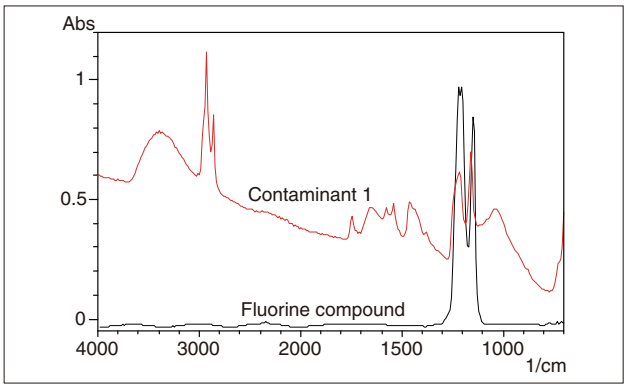


Fig. 7.3.4 Search Result 1

7.3 Analysis of Contaminant Adhering to Frozen Pizza (2) - FTIR/EDX/EPMA

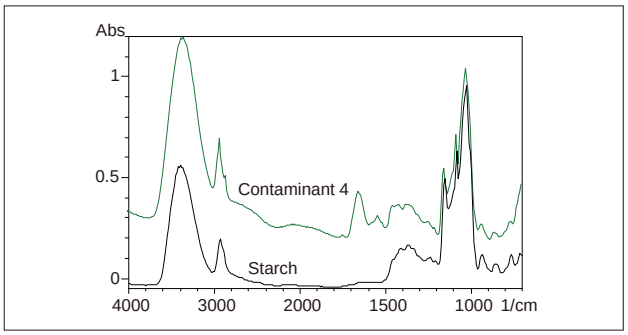


Fig. 7.3.5 Search Result 2

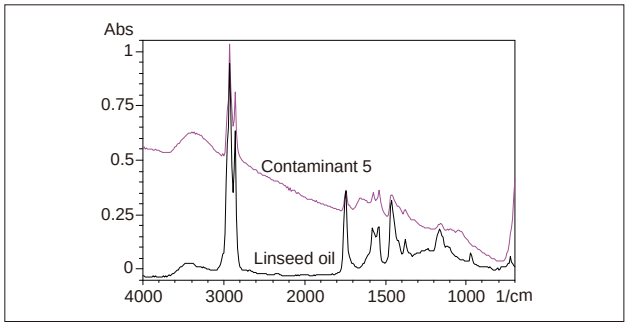


Fig. 7.3.6 Search Result 3

As can be seen from the respective search results, there were search hits on a fluorine compound, starch, and linseed oil. Of these, the starch and linseed oil are expected components of the pizza dough. The fluorine compound is an industrial product, and since it is also used in cooking utensils, it is apparently a contaminant derived from an external source.

■Analysis by EDX

Analysis of the intact contaminant adhering to the frozen pizza was conducted using a 3 mm analysis diameter. Fig. 7.3.7 shows the qualitative results comparing the normal and contaminated parts of the frozen pizza. In addition, a comparative profile of the normal and contaminated sites of Fig. 7.3.7 was calculated, and quantitative analysis of the detected elements was conducted by the FP method. The obtained results are shown in Table 7.3.2.

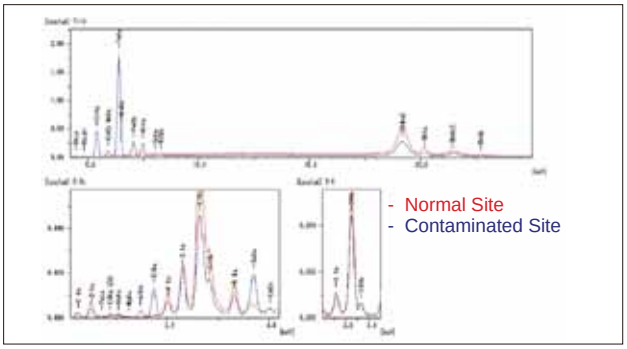


Fig. 7.3.7 6C-92U Qualitative Results for Contaminated and Normal Sites

Table 7.3.2 Quantitative Results for Contaminated Site by FP Method (%)

Fe	Cr	Ni	Si	Ca	Al	Ba	Mn
59.42	12.07	8.97	8.91	4.69	3.55	1.72	0.79

From the results shown in Fig. 7.3.7 in which Fe, Cr and Ni were detected in the contaminant as principal components, and considering the quantitative results, the contaminant is presumed to consist of stainless steel (SS). Further, since Al, Si, Ca and Ba were also detected, it is possible that a ceramic or other pigment-containing material may also be included. Since Na, P, S, Cl, K and Ca were also detected at the uncontaminated site, these are assumed to be of food origin. As for the F that is derived from the fluorine compound and was detected by FTIR, it was not clearly detected here, probably due to the small relative mass within the analysis radius and the overlap with the FeLα line.

■Analysis by EPMA

After scraping off a portion of the contaminant and coating its surface with gold, we conducted mapping of each element within a micro-region measuring 400 × 400 µm. Fig. 7.3.8 shows the mapping results for the principal elements that were detected. The SS constituents Fe and Cr that were detected by EDX were detected over a wide range. In addition, the F that was detected by FTIR was detected in a localized perimeter. Thus, the results are consistent with the obtained FTIR and EDX results.

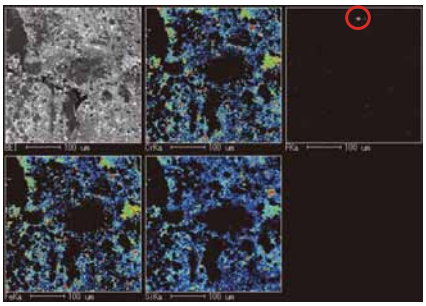


Fig. 7.3.8 Results of Elements Mapping for Contaminant Part

■Conclusion

Complex qualitative analysis using FTIR, EDX and EPMA was conducted to identify a contaminant adhering to the surface of frozen pizza, and the results indicated detection of SS constituents and a fluorine compound. EPMA mapping results indicated that the SS constituents (Fe, Cr) were scattered over a region of approximately 10 µm. On-site verification in the actual manufacturing process is essential to identify the source of contamination, but from the photograph of Fig. 7.3.1, the contaminant appears to be some type of burnt substance. The above analysis suggested that the contaminant consists of burnt cooking oil mixed with a fluorine compound originating from a cooking utensil or manufacturing machine, in addition to SS powder.

7.4 Introduction of a Search System for Contaminants in Tap Water (1) - FTIR/EDX

■Explanation

Various contaminants, both organic and inorganic, can be present in tap water. It is therefore necessary to identify not only contaminants that are organic, but also those that are inorganic in nature. Without information on both of these types of contaminants, adequate determination is not possible. Here, we present the determination of organic and inorganic contaminants in tap water using both infrared spectroscopy (FTIR) for analysis of organic substances, and X-ray fluorescence analysis (EDX) for inorganic elements.
we present the analysis of these two types of contaminants in tap water and introduces how a tap water contaminant search system can be utilized for identification of the substances.

■Contaminants Found in Tap Water

Contaminants due to foreign matter in tap water can originate from a variety of sources, including the tap water system materials themselves, minerals, and microorganisms. In particular, major sources of contamination include rubber and metallic components from seals that have degraded due to aging of the water supply equipment. These types of contaminants should be quickly identified to allay the concerns of users. A combination of FTIR and EDX analysis can effectively provide useful information for elucidating the identity of a foreign substance. FTIR analysis can be used to identify organic compounds and some inorganic compounds, while EDX analysis can be used to obtain qualitative and quantitative information for such substances as iron rust and scale (calcium and magnesium deposits), which are difficult to identify from infrared spectra. In addition, this approach is effective in narrowing down and identifying the possible contaminant candidates when seals contain the same principal components (organic substances) but different additives. Another strong advantage is that the sample need only be about 1 mm in size for analysis by either FTIR or EDX.

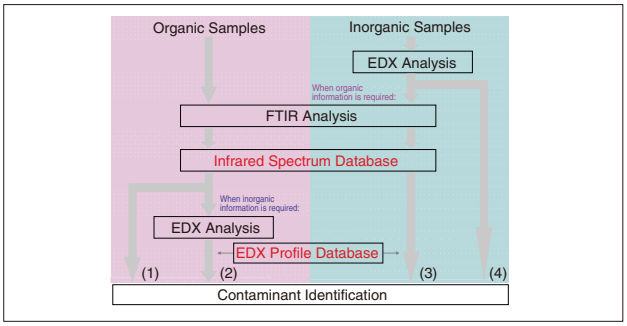


Fig. 7.4.1 Procedure of Contaminant Analysis by Tap Water Contaminant Search System

■The Features of Tap Water Contaminant Search System

The tap water contaminant search system comprises two databases, an infrared spectrum database and an EDX profile database. The infrared spectrum database includes the infrared spectra of contaminants collected from tap water, in addition to the infrared spectra of commercially available tap water system maintenance parts. Since searches are conducted using a database created from actual contaminants found in tap water, the search suitability rate is quite good. In addition to qualitative results, the color, shape, hardness, presence or absence of metallic luster, and measurement technique pertaining to the contaminant materials are also recorded in the database. Furthermore, the elemental information obtained from EDX analysis results is also stored in this database, thereby permitting details to be viewed using the EDX profile database. In addition to the qualitative and quantitative information, the EDX profile database also includes photographs of the samples. The procedure for identifying contaminants using the tap water contaminant search system is shown in Fig. 7.4.1. This sequence is used for analysis regardless of the category of the system where the contaminant was discovered. Here, we used the flow chart scheme to analyze contaminants in tap water.

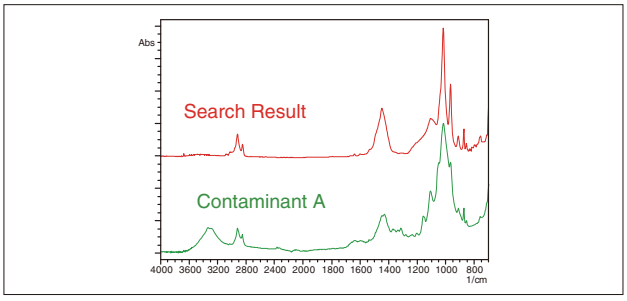
■Analysis of Organic Material

First, observation of contaminant A that was detected in tap water revealed a lusterless, black-colored material, suggesting that it was an organic substance. In the case of organic contaminants, following the procedure for "Organic Samples" shown in Fig. 7.4.1, FTIR analysis was conducted first. Contaminants found in tap water are typically visible, so it is simple to conduct measurement using a single reflection ATR accessory. The analytical conditions are shown in Table 7.4.1, and a photograph of the instrument used is shown in Fig. 7.4.2. The search results obtained using the infrared spectrum database are shown in Fig. 7.4.3. From this, it is clear that components of the spectrum of the contaminant matches well to a "Discharge pipe packing" (principal component is SBS).

Table 7.4.1 Instrument and Analytical Conditions

Instrument	: IRAffinity-1, MIRacle10 Ge, Diamond
Resolution	: 4.0 cm^{-1}
Accumulation	: 40
Apodization	: Happ-Genzel
Detector	: DLATGS

Fig. 7.4.2 Photograph of IRAffinity-1 with MIRacle 10 ATR Accessory Attached



Details of infrared spectrum database search result:
"Discharge pipe 32 packing_Gray_Inside"
Materials: Styrene butadiene styrene (SBS), Calcium carbonate ($CaCO_3$), Magnesium silicate (TALC, $3Mg_4SiO_2H_2O$)
Major elements: Cl, Ca, Si, Mg, S, Zn, Ti
Color: Gray Shape: Resin/Ring-shaped Hardness: Soft
Metallic luster: No Technique: ATR (Diamond)

Fig. 7.4.3 Infrared Spectra and Search Results for Contaminant A

7.4 Introduction of a Search System for Contaminants in Tap Water (2) - FTIR/EDX

Next, as indicated in procedure (2) of Fig. 7.4.1, EDX analysis was conducted to obtain element information, and the "Discharge pipe packing" determined in the FTIR analysis result was matched against the EDX profile database. The profile used for matching is shown in Fig. 7.4.4. Primarily, the elements Ca, Si, and Mg were clearly detected. These results clearly support the presence of calcium carbonate and magnesium silicate (talc), as shown in details of infrared spectrum database search result-Fig. 7.4.3. Here, when the main component is an organic substance (in this case SBS) as identified in a search of the infrared spectra, the quantitative results obtained from the EDX profile database are calculated using the FP method.

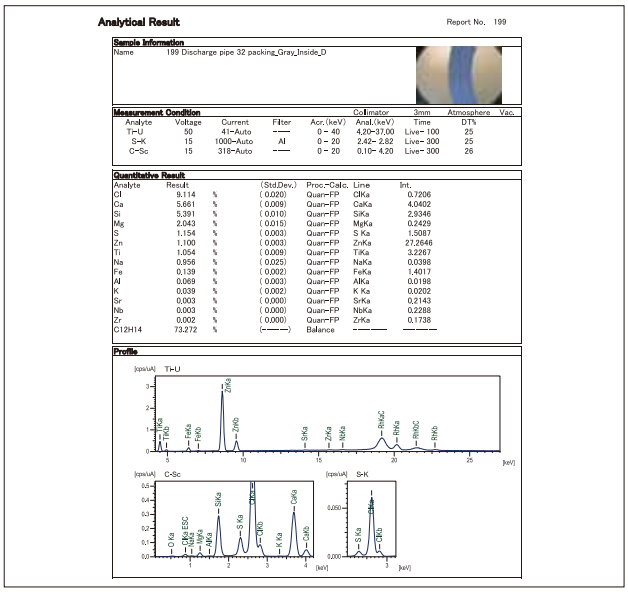


Fig. 7.4.4 Qualitative and Quantitative Results for "Drain Fitting Seal" by EDX

■Analysis of Inorganic Materials

Next, observation of contaminant B in tap water revealed a powder sample with metallic luster, suggesting that contaminant B was an inorganic material. Inorganic contaminants are first analyzed by EDX, as indicted in the procedure of Fig. 7.4.1 for Inorganic Samples. The analytical conditions are shown in Table 7.4.2, and a photograph of the instrument used is shown in Fig. 7.4.5. The EDX analysis results are shown in Fig. 7.4.6. The principal component of this contaminant was detected as Fe, and is assumed to consist of iron rust based on the quantitative results. In addition, contaminant B was measured by the FTIR single reflection ATR method, and the infrared spectrum database was used to conduct a search, as outlined in procedure (3) of Fig. 7.4.1. The infrared spectrum search results are shown in Fig. 7.4.7. These results indicated a similarity of the contaminant to iron rust. Also, this similarity is supported by the "Iron Rust_3" hit in the FTIR search result using the EDX profile database. This analysis shows that tap water contaminants can be identified more accurately using the EDX profile database together with the infrared spectrum database.

Table 7.4.2 Instrument and Analytical Conditions

Instrument	: EDX-800HS
X-Ray Tube	: Rh target
Filter	: #1 (for Cl)
Tube Voltage	: 15 [kV] (C - Sc), 50 [kV] (Ti - U)
Tube Current	: Auto
Atmosphere	: Vacuum
Measurement Diameter	: 1 [mmφ]
Measurement Time	: 100 [sec] (Ti-U), 300 [sec] (C-Sc), 300 [sec] (Cl)
Dead Time	: Max 25 [%]

Fig. 7.4.5 Photograph of EDX-800HS Energy Dispersive X-Ray Fluorescence Spectrometer

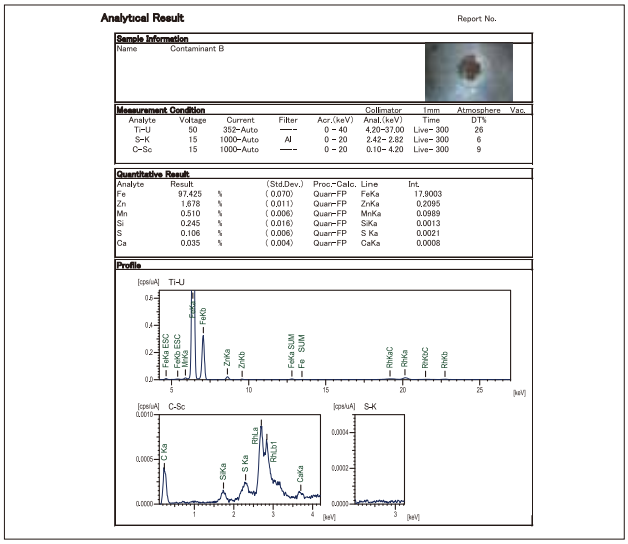
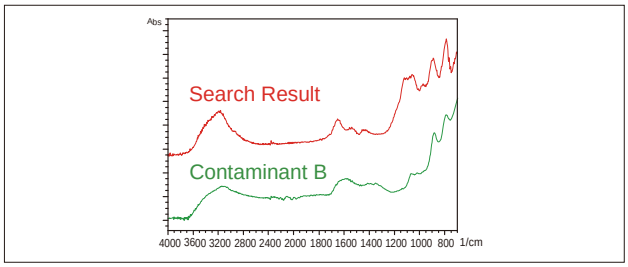


Fig. 7.4.6 Qualitative and Quantitative Results for Contaminant B by EDX



Details of infrared spectrum database search result: "Iron rust_3" Materials: Iron (III) oxide hydroxide ($Fe(OH)_3$) Major elements: Fe, S, Si, P Color: Yellow Shape: Sand-shaped Hardness: Soft Metallic luster: No Technique: ATR (Ge)

Fig. 7.4.7 Infrared Spectra and Search Results for Contaminant B

■Conclusion

Here, we introduced a tap water contaminant search system using FTIR analysis and EDX analysis. Even when a commercially available product is provided with information about materials that are included, information related to additives is often not known, making it difficult to identify such materials as rubber and plastics. However, the infrared spectrum database prepared here includes qualitative information related not only to principal components but also to additives, in addition to elemental information detected by EDX. Also included is a sample database containing information collected from actual contaminants, as well as cross-section and surface measurements of seals, which have a relatively high detection rate in tap water. Thus, this system can be utilized as a contaminant analysis tool not only for tap water-related utilities, but across a wide range of fields, such as environmental (including contract analysis), petrochemical, and food fields.

7.5 Identification of Contaminants (Animal Hair) (1) - MCE

■Explanation

If a contaminant is discovered during the process of manufacturing foods, medicines, and cosmetics, etc., it is essential to identify the contaminant not only to clarify the cause or source of the contamination but for establishing a standard operating procedure to prevent the recurrence of such contamination as required in hygiene control. Presumptive inspection of contaminants is typically conducted by microscopic observation, but since this requires specialized knowledge and experience, it is difficult for inexperienced individuals to make a clear assessment and to determine the source of contamination, human or animal. Especially, with respect to animal hair, a very common contaminant, it is extremely difficult to determine the type of contamination by visual inspection. Reliable methodology is therefore required to solve this problem. The Aichi Industrial Technology Institute Food Research Center has developed a DNA testing method for identifying the type of animal associated with specific hair contaminants(*). The animal species that can now be detected reliably with this method include 6 types of farm animals (cow, pig, chicken, horse, sheep, and goat), 3 types of pets (dog, cat, and rabbit), and 3 types of rodents (sewer rat, black rat, and house mouse), for a total of 12 animal types. Here we introduce examples of analysis of animal types by DNA testing using a combination of the abovementioned method together with Shimadzu's MCE-202 MultiNA microchip electrophoresis system.

■Experimental Procedure

The DNA extraction method and PCR conditions used were in accordance with the "Animal Hair DNA Testing Protocol"(*) developed by the Aichi Industrial Technology Institute Food Research Center. The samples used for PCR consisted of DNA samples of cow, pig, chicken, horse, sheep, goat, dog, cat, rabbit, sewer rat, black rat, and house mouse that were kindly provided by the Aichi Industrial Technology Institute Food Research Center. In addition to these, DNA was also extracted from the hair of 4 breeds of dog (Shiba, chihuahua, corgi, and Afghan hound), a cat (Norwegian forest cat), and a rabbit (Lionhead rabbit). Following PCR, the amplified DNA was subjected to size analysis using the MultiNA. The animal types were identified from the analysis results (See procedure flowchart, Fig. 7.5.1).

* Animal Hair DNA Testing Protocol
(Aichi Industrial Technology Institute Food Research Center)
http://www.pref.aichi.jp/cmsfiles/contents/0000016/16149/protocol0821.pdf
Patent Application Number: Special Application 2007-240023
Invention Name: Animal Identification Primer Set and Primer Kit
The Primer Set is manufactured and marketed by Bex Co., Ltd. with the permission of Aichi Prefecture.
http://www.bexnet.co.jp

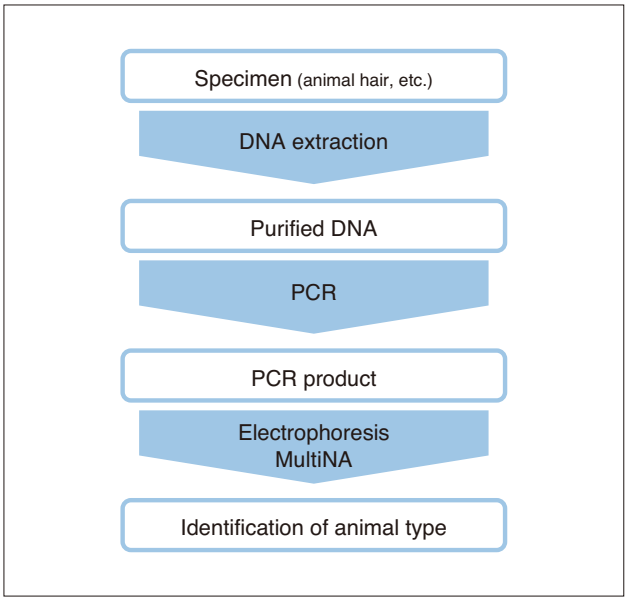


Fig. 7.5.1 Experimental Procedure for Identification of Contaminants (Animal Hair) Using DNA Testing

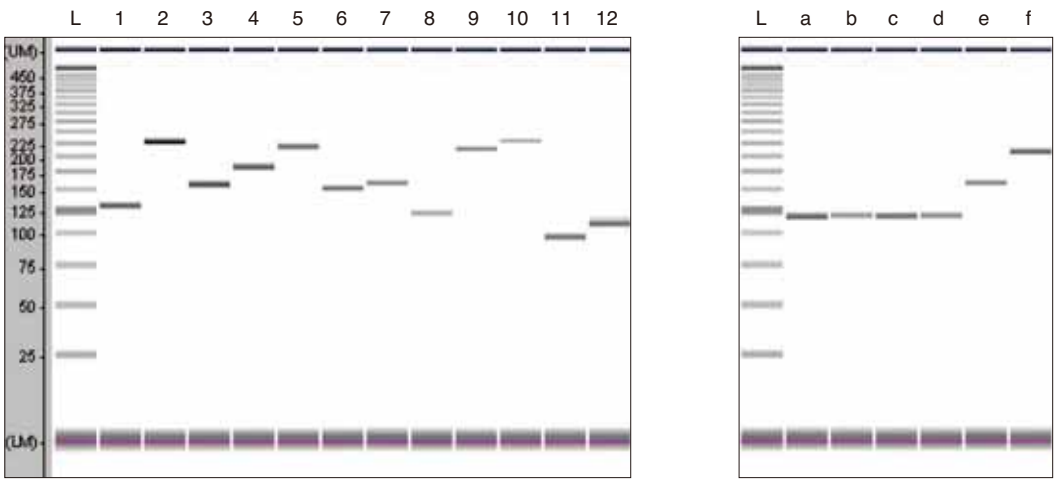
■Reagents/Kits

* QIAGEN Fast Cycling PCR Kit
  (Qiagen) 203743
* Primer Sets for identification of animals
  (each 12 types) (Bex)
* QIAamp DNA Micro Kit
  (Qiagen) 56304
* DNA-500 kit
  (Shimadzu Corp.) P/N: 292-27910-91
* GelStar® Nucleic Acid Stain
  (Takara Bio) F0535
* 25 bp DNA Ladder
  (Invitrogen) 10597-011

7.5 Identification of Contaminants (Animal Hair) (2) - MCE

■Results

Fig. 7.5.2 shows the analysis results for the PCR products of the 12 animal types (cow, pig, chicken, horse, sheep, goat, dog, cat, rabbit, sewer rat, black rat, and house mouse) and dog breeds (Shiba, chihuahua, corgi, and Afghan hound), cat (Norwegian forest cat), and rabbit (Lionhead rabbit). Characteristic lengths of PCR products (cow (137 bp), pig (230 bp), chicken (159 bp), horse (183 bp), sheep (224 bp), goat (160 bp), rabbit (167 bp), dog (122 bp), cat (220 bp), sewer rat (237 bp), black rat (102 bp), and house mouse (116 bp)) were amplified for the 12 animal types, respectively. The DNA of the 12 types of animals was detected from the electrophoresis results of 1 to 12 (Fig. 7.5.2). In addition, with the electrophoresis results of a to f (Fig. 7.5.2), DNAs of lengths which can be used to identify the animal types were also detected from the respective hair of dog (Shiba, chihuahua, corgi, and Afghan hound), rabbit (Lionhead rabbit), and cat (Norwegian forest cat). By combining the use of the Primer Sets and the MultiNA microchip electrophoresis system as described in this analysis example, contaminants can be identified from minute quantities of biological substances such as hair, etc., often discovered in manufacturing processes and products.



L : 25 bp DNA Ladder
1 : cow
2 : pig
3 : chicken
4 : horse
5 : sheep
6 : goat
7 : rabbit
8 : dog
9 : cat
10 : sewer rat
11 : black rat
12 : house mouse

L : 25 bp DNA Ladder
a : dog (chihuahua)
b : dog (corgi)
c : dog (Afghan hound)
d : dog (Shiba)
e : rabbit (Lionhead)
f : cat (Norwegian forest cat)

Fig. 7.5.2 Analytical Results of PCR Products

7.6 Analysis of Odor in Frozen Shrimp (1) - GC/MS

■Explanation

The smell of food is an important characteristic related to its taste, aroma, and freshness. The headspace technique, in which the gas released during heating of the sample is analyzed, has typically been used in the aroma analysis of foods. The headspace technique usually relies on heating of the sample to transfer the target substances to the vapor phase. However, in the case of perishable foods, there is always a danger that heating will cause sample degeneration. Although the headspace technique can be conducted using a lower heating temperature, this approach would normally result in reduced sensitivity. An alternate approach when analyzing samples which could be affected by thermal degradation involves concentrating the volatilized gas at or near room temperature using the thermal desorption technique. Here we introduce an example of analysis of trace quantities of substances volatilized from frozen shrimp at room temperature.

■Analysis Outline

1. Sampling
   Volatile substances were collected.
2. Sample introduction
   Volatile substances were introduced into GC/MS.
3. Data analysis
   3.1 Qualitative analysis
   Volatile substances were identified from their mass spectra.
   3.2 Quantitative analysis of the generated gas
   For each substance identified, a calibration curve was generated using standard samples of known concentration. The volatilized compounds in the shrimp samples were calculated using the calibration curves.

■1. Sampling Method

The frozen sample (5 g) was enclosed in a container and set aside for 30 min to defrost at room temperature. Nitrogen gas was then passed through the container at 100 mL/min. A total of 500 mL of the expelled gas was passed through the collection tube containing adsorbent packing for trapping the target substances, thereby concentrating the volatile odor substances. Since this analysis targets trimethylamine, a collection tube packed with CarbotrapB+Carboxen1000 (SHIMADZU PN 223-57474-91) was used. TENAX-TA, which is a frequently used packing for thermal desorption analysis, was not used in this case due to its weak retention of trimethylamine. Weak retention is cause for concern due to the possible breakthrough and loss of the analyte. The type of collection tube must be selected according to the chemical properties of the analytes.

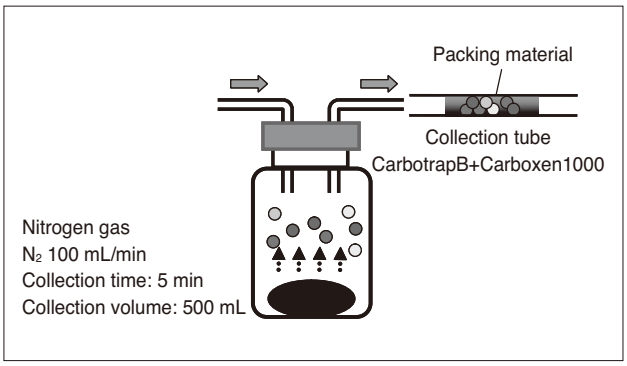


Fig. 7.6.1 Schematic Diagram of Sampling Method

■2. Sample Introduction

Analysis of the volatile odor substances of frozen shrimp was conducted using the thermal desorption technique. The thermal desorption technique uses a collection tube which is connected to the instrument; the collection tube, containing volatile substances collected from the sample, is thermally desorbed and introduced into the GC/MS. Since the sample width (bandwidth) of the volatile substances widens at the time of desorption from the collection tube, typically, a secondary trap tube is installed and the volatile substances are cooled and re-concentrated to narrow the bandwidth, thus achieving higher chromatographic resolution. The analytical conditions are shown on next page. Sample introduction when using thermal desorption is as illustrated in the schematic diagram of Fig. 7.6.2.

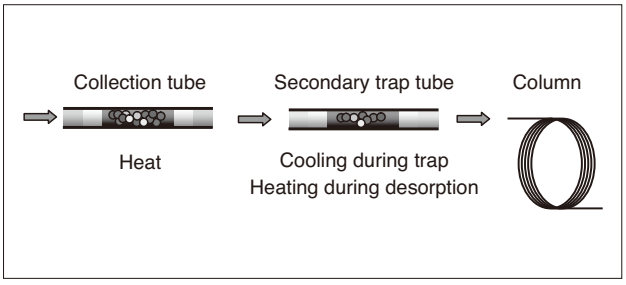


Fig. 7.6.2 Schematic Diagram of Thermal Desorption

7.6 Analysis of Odor in Frozen Shrimp (2) - GC/MS

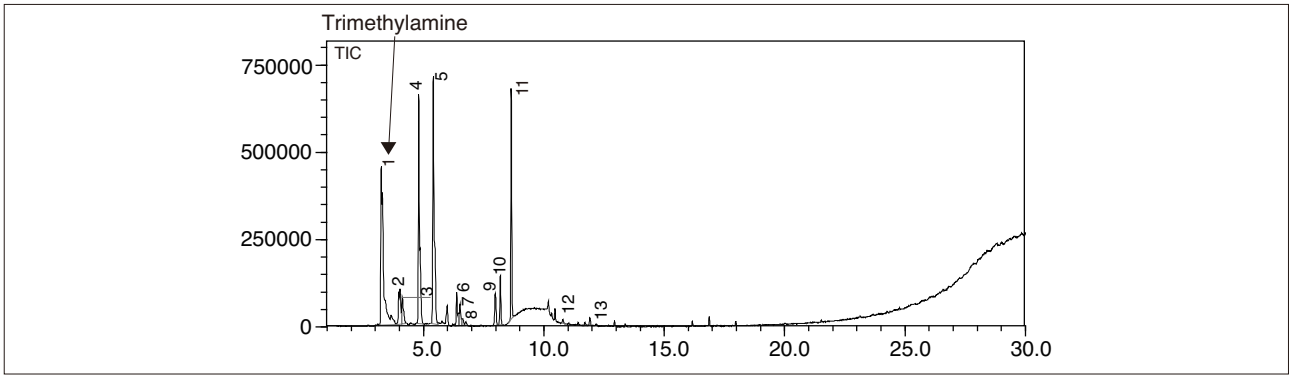


Fig. 7.6.3 Total Ion Current Chromatogram of Frozen Shrimp

3. Data Analysis

3.1 Qualitative Analysis

The total ion current chromatogram obtained from analysis of the frozen shrimp is shown in Fig. 7.6.3. The principle peak was identified by conducting a similarity search with the NIST08 Mass Spectral Library. The identification results are shown in Table 7.6.1. Trimethylamine, carbon disulfide and dimethyl sulfide, etc. were confirmed.

Analytical Conditions

-TD-

Instrument	: TD-20
Desorp Temp.	: 250 ˚C
Desorp Flowrate	: 60 mL/min
Desorb Time	: 10 min
1st Trap Tube	: CarbotrapB+Carboxen1000 (SHIMADZU PN223-57474-91)
2nd Trap	: TENAX-TA (SHIMADZU PN223-54144-91)
Trap Low Temp.	: -20 ˚C
Trap High Temp.	: 250 ˚C
Valve Temp.	: 230 ˚C
Line Temp.	: 250 ˚C
IF Temp.	: 230 ˚C
Instrument	: GCMS-QP2010 Plus

-GC-

Column	: RESTEK Stabilwax (60 m × 0.32 mm I.D. df = 0.5 µm)
Column Temp.	: 40 ˚C (2 min) – 8 ˚C/min – 250 ˚C (15 min)
Carrier Gas	: He (Constant Linear Velocity Mode)
Linear Velocity	: 36 cm/s
Injection Method	: Split
Split Ratio	: 1:15

-MS-

Interface Temp.	: 230 ˚C
Ion Box Temp.	: 200 ˚C
Ionization Method	: EI
Scan Range	: *m/z* 35 - 550
Scan Interval	: 0.5 s

Table 7.6.1 Results of Similarity Search

Peak	compound name
1	Trimethylamine
2	Carbon disulfide (CS_2)
3	Dimethyl sulfide
4	Acetone
5	Tetrahydrofuran (THF)
6	Isopropyl alcohol
7	Chloromethoxymethane
8	Ethanol
9	Acetonitrile
10	Chloroform
11	Toluene
12	1-Butanol
13	Pyridine

3.2 Quantitative Analysis of Generated Gas

Quantitative analysis was conducted to determine the amount of generated gas associated with the detected peak number 1, trimethylamine (retention time at approximately 3 minutes). A trimethylamine standard substance was diluted with methanol, and quantities corresponding to 0.1, 0.3 and 1 µg were added to the collection tube. Analyses were then conducted using the same procedure as with the actual sample. Fig. 7.6.4 shows the calibration curve generated using the area values of the mass chromatogram of *m/z* 58. For the quantitative calculation results, quantities added to the collection trap were assumed to correspond to the collected headspace volume of 500 mL.

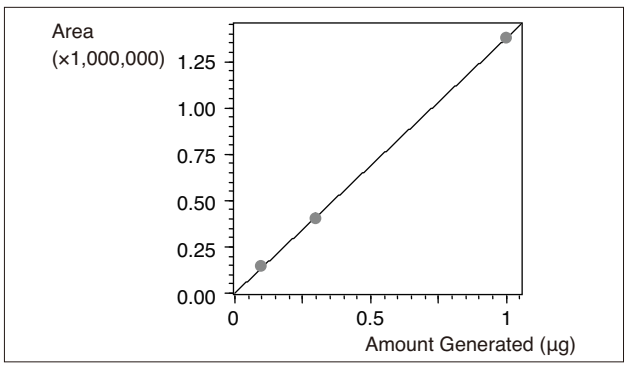


Fig. 7.6.4 Calibration Curve of Trimethylamine (*m/z* 58)

8. Food Properties

8.1 Melting of Chocolate - TA

■Explanation

The melting process of various edible fats and oils is measured by DSC. Six types of crystals exist in cocoa oil, a component of chocolate. Of these, V type crystal is said to possess good thermal stability. Since V type crystal melts at about 34 °C, DSC measurement can be used to know the condition of V type crystal contained in a particular sample of chocolate. Fig. 8.1.1 shows a DSC curve of the chocolate sample heated at 3 °C/min. Fig. 8.1.2 shows a DSC curve of the same chocolate sample, reheated after cooling the melted sample to -50 °C to harden it. It is evident that the peak at 30.4 °C has completely disappeared.

■Analytical Conditions

Instrument	: DSC-60
Sample	: Chocolate
Sample Amount	: 22.87 mg
Atmospheric Gas	: Nitrogen
Gas Flowrate	: 30 mL/min
[Temperature Program]	
Heating Rate	: 3 °C/min

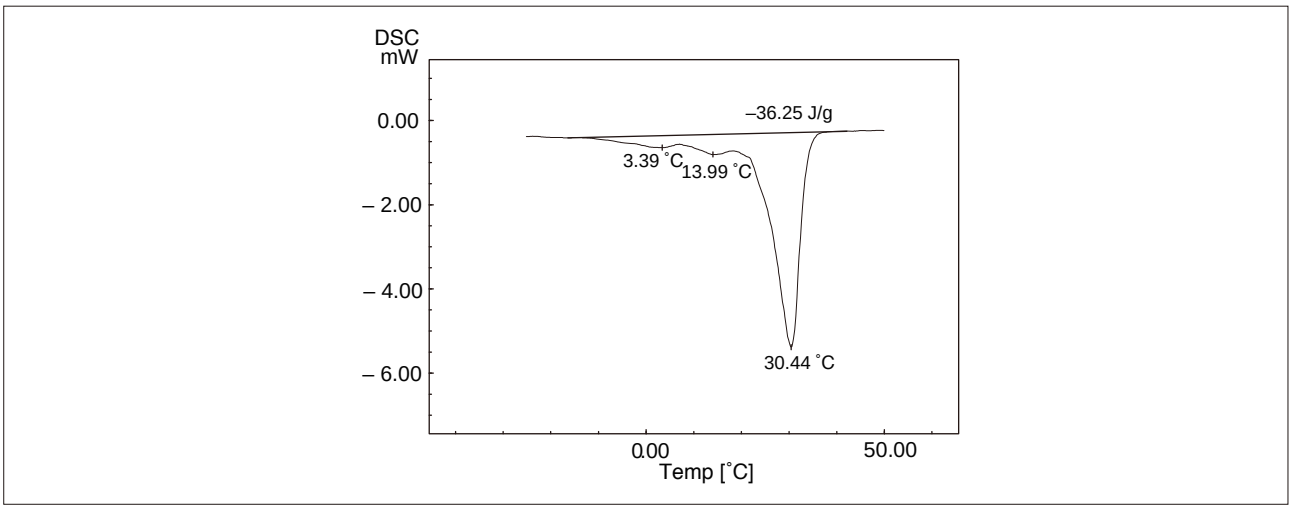


Fig. 8.1.1 Chocolate Measurement (1st time)

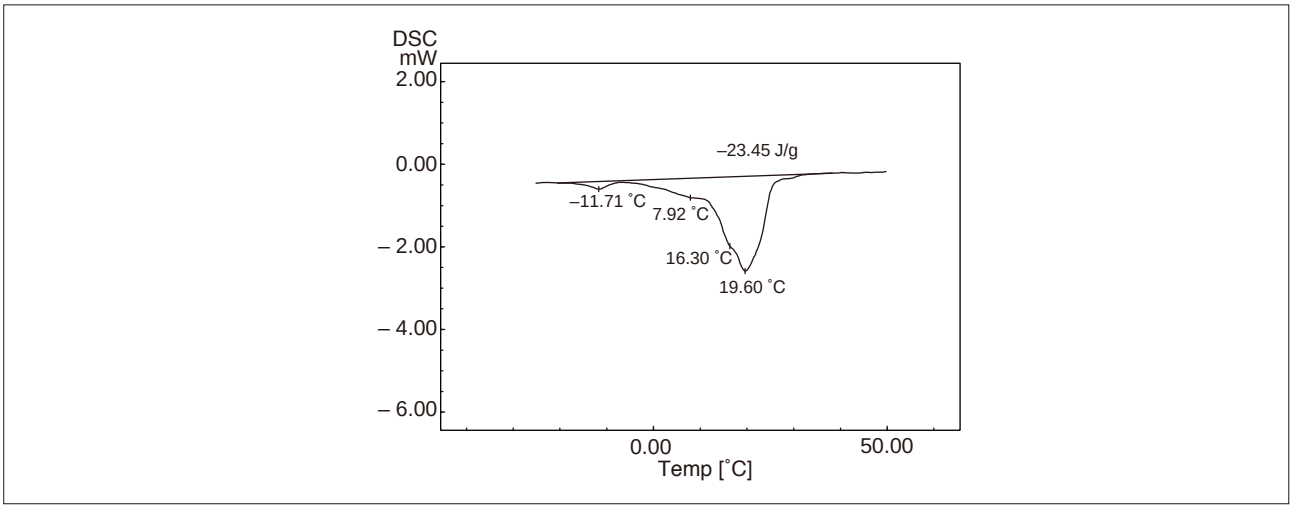


Fig. 8.1.2 Chocolate Measurement (2nd time)

8.2 Gelatinization of Starch - TA

■Explanation

Starches gelatinize when heated with water. The gelatinization reaction can be analyzed by DSC because it is accompanied by endothermic reaction. Here we conducted measurement of flour starch (17.4 %).

■Analytical Conditions

Instrument	: DSC-60
Sample	: Flour
Sample Amount	: 4.21 mg
Atmospheric Gas	: Nitrogen
Gas Flowrate	: 30 mL/min
[Temperature Program]	
Heating Rate	: 5 °C/min

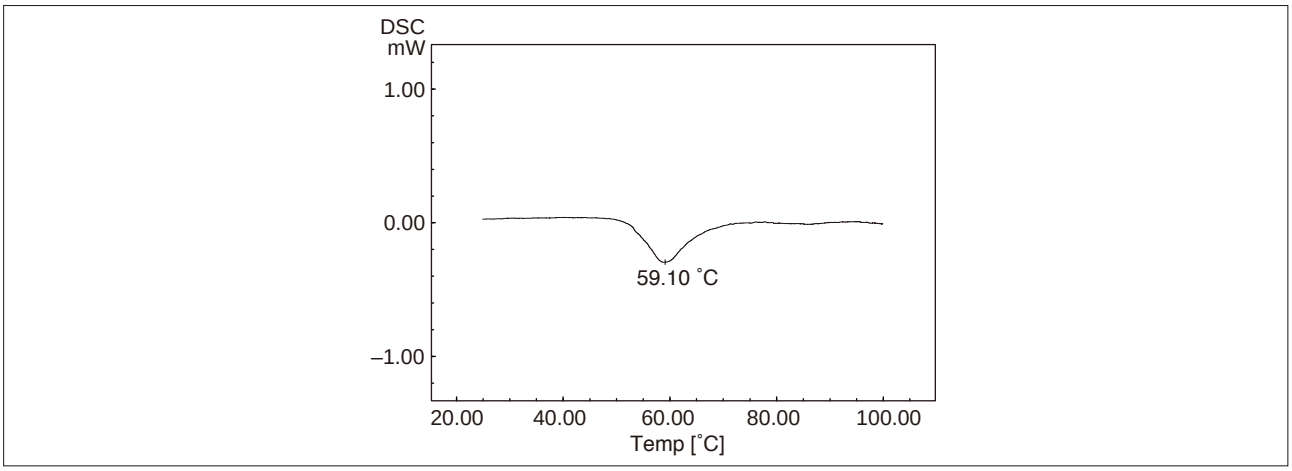


Fig. 8.2.1 Gelatinization Temperature of Flour (17.4 %)

■Explanation

Here we conducted measurement of corn starch (19.9 %). It is known that when sucrose and salt are added to starch, the gelatinization temperature changes.

■Analytical Conditions

Instrument	: DSC-60
Sample	: Corn
Sample Amount	: 4.97 mg
Atmospheric Gas	: Nitrogen
Gas Flowrate	: 30 mL/min
[Temperature Program]	
Heating Rate	5 °C/min

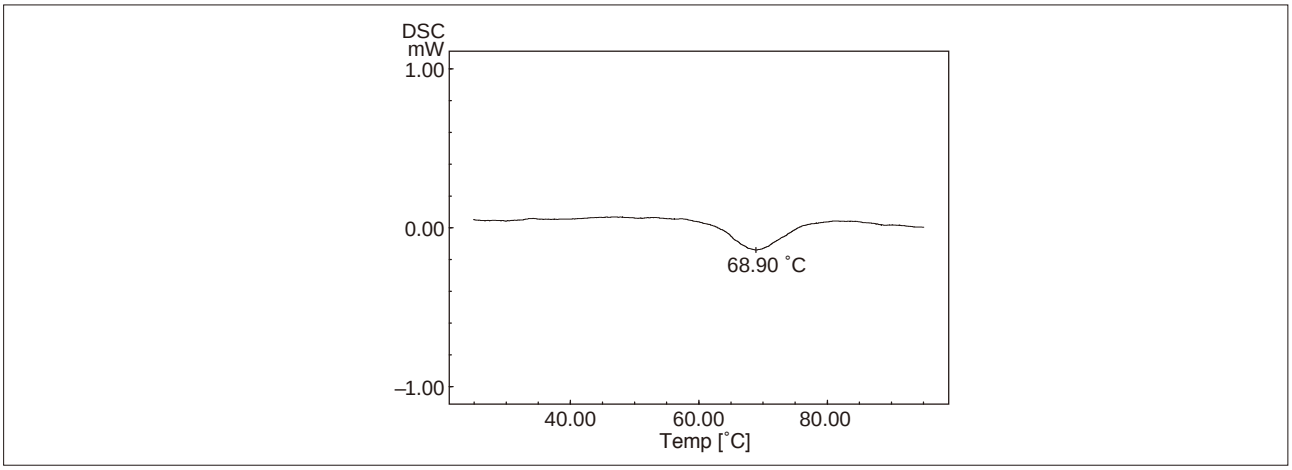


Fig. 8.2.2 Gelatinization Temperature of Corn (19.9 %)

8.3 DSC Measurement of Liquor - TA

■Explanation

Whiskey was sealed in a hermetic cell and measured by DSC. The exothermic peak at -74.2 °C is due to crystallization. The peak at -65.9 °C shows the melting of ethanol, -49.8 °C the eutectic point of ethanol and water, and -25.6 °C the melting of water. The height of the eutectic peak varies depending on the storage period of the malt.

■Analytical Conditions

Instrument	: DSC-60
Sample Name	: Whiskey
Sample Weight	: 13.7 mg
Atmospheric Gas	: Nitrogen
Gas Flowrate	: 30 mL/min
[Temperature Program]	
Heating Rate	: 10 °C/min

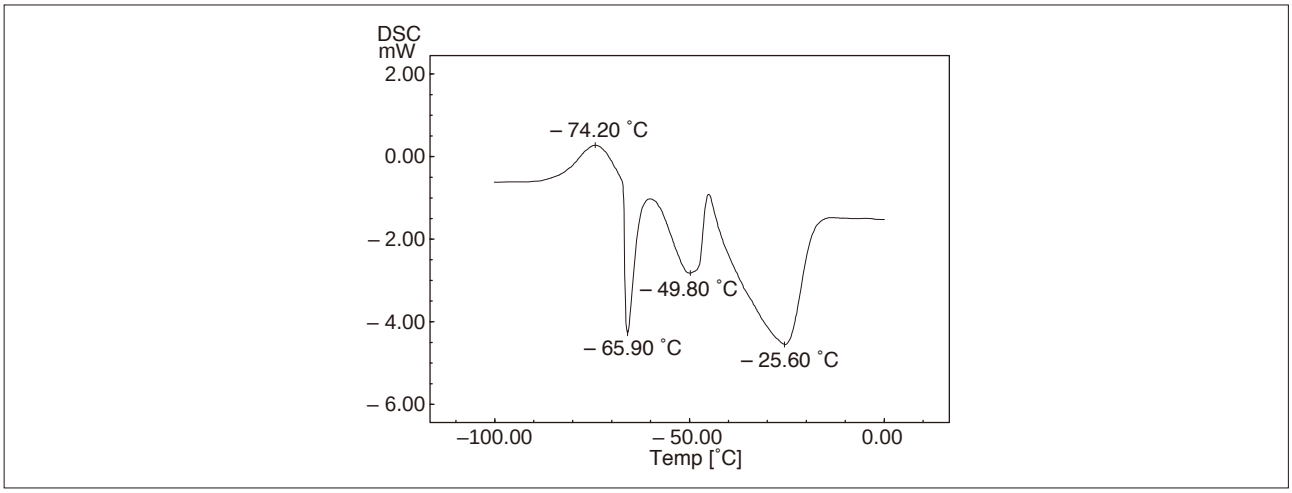


Fig. 8.3.1 DSC Curve for Whiskey

■Explanation

Brandy was sealed in a hermetic cell and measured by DSC.

■Analytical Conditions

Instrument	: DSC-60
Sample Name	: Brandy
Sample Weight	: 9.11 mg
Atmospheric Gas	: Nitrogen
Gas Flowrate	: 30 mL/min
[Temperature Program]	
Heating Rate	: 10 °C/min

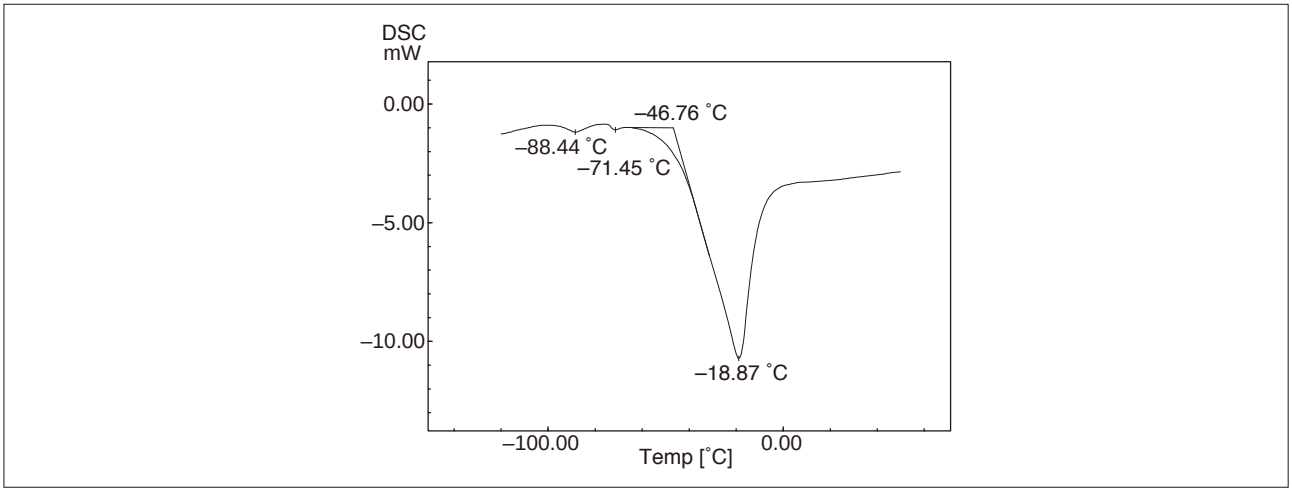


Fig. 8.3.2 DSC Curve for Brandy

8.4 DSC Measurement of Fish Meat - TA

■Explanation

DSC analyzed the freshness of carp meat. With the most fresh carp meat, an exothermic peak at 42.8 °C and endothermic peaks at 56.3 °C and 75.8 °C were observed (Fig. 8.4.1). With carp meat after 6 hours, a minute exothermic peak at 37.0 °C and endothermic peaks at 54.3 °C and 75.7 °C were observed (Fig. 8.4.2). With carp meat after 24 hours, endothermic peaks at 42.3 °C, 55.1 °C and 73.1 °C were observed but no exothermic peak was observed (Fig. 8.4.3). The endothermic peaks at 42 °C and around 73 °C are due to the denaturation of myosin and actin, respectively. The exothermic peak at around 40 °C corresponds to the shrinkage of myosin and actin caused by ATP remaining in the fish meat. The amount of ATP remaining varies with the storage period.

■Analytical Conditions

Instrument	: DSC-60
Sample Name	: Carp Meat
Sample Weight (Fig. 8.4.1)	: 25.4 mg
(Fig. 8.4.2)	: 24.5 mg
(Fig. 8.4.3)	: 26.5 mg
Atmospheric Gas	: Nitrogen
Gas Flowrate	: 30 mL/min
[Temperature Program]	
Heating Rate	: 5 °C/min

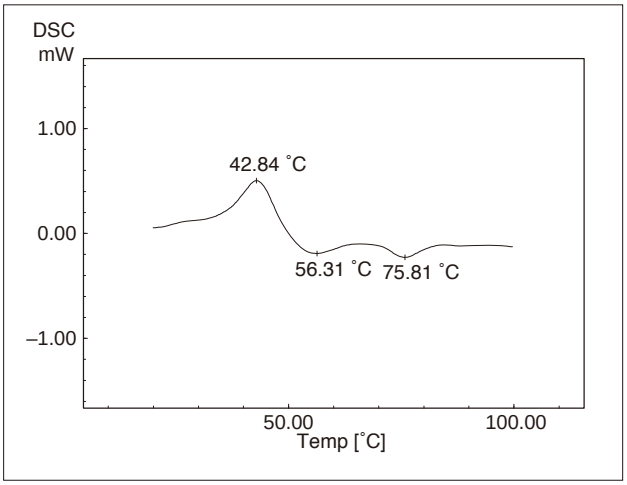


Fig. 8.4.1 DSC curve for Fresh Fish Meat

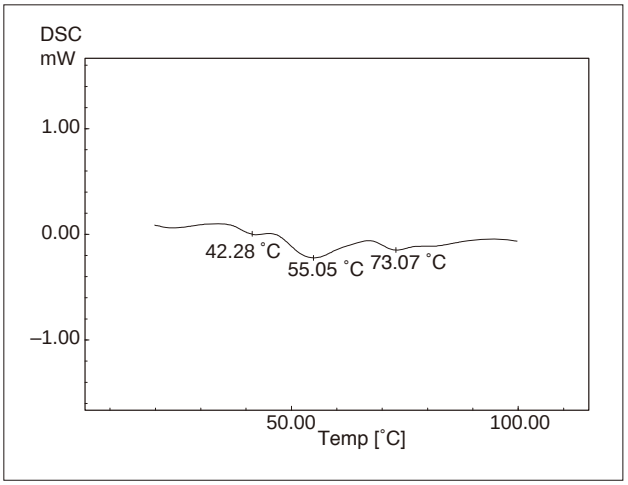


Fig. 8.4.3 DSC Curve for Fish Meat after 24 Hours

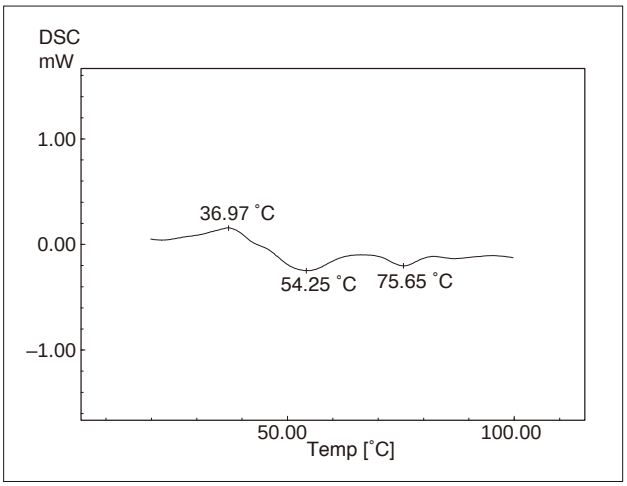


Fig. 8.4.2 DSC Curve for Fish Meat after 6 Hours

8.5 Protein Denaturation and Texture Analysis for Chicken (1) - TA

■Explanation

The deliciousness of food is greatly influenced by taste due to chemical interactions with the tongue for the basic tastes of sweetness, saltiness, acidity, bitterness, and umami (also referred to as savoriness), in addition to the physical sensations of hardness and softness and juiciness sensed by the teeth. In particular, texture, which includes the sensations of juiciness and softness, is an important factor related to the deliciousness of fried foods, such as fried chicken. Here we conducted an overall evaluation of texture in fried chicken heated over different time periods. DSC was to measure protein denaturation, and a precision universal testing machine was used to measure the hardness and elasticity.

■Protein Denaturation of Chicken

Chicken breast seasoned with spices was placed in an aluminum sealed cell, and heated at rate of 10 °C/min. Endothermic peaks were seen at 57 °C, 64 °C and 78 °C. It is presumed that the endothermic peaks correspond to denaturation of myocin at 57 °C, connective tissue at 64 °C, and actin at 78 °C.

■Protein Denaturation at Different Heating Times

After confirming that the temperature at the center of frying chicken breasts was 70 °C, the chicken breasts were kept warm for 0 min, 1 hour, 2 hours, and 4 hours, respectively, at which times the chicken breasts were immediately frozen by shock freezing. Before conducting DSC measurement of the frozen samples, they was defrosted and placed in an aluminum sealed cell, and heated at a rate of 10 °C/min up to 100 °C. An endothermic peak due to denaturation of the protein actin is seen within one hour of heat retention. This means that un-denatured protein still remains. On the other hand, when heat retention continues beyond 2 hours, no endothermic peaks are seen, and the softness is lost. Thus, this correlates to the experience that the longer heat retention continues, the dryer the texture becomes.

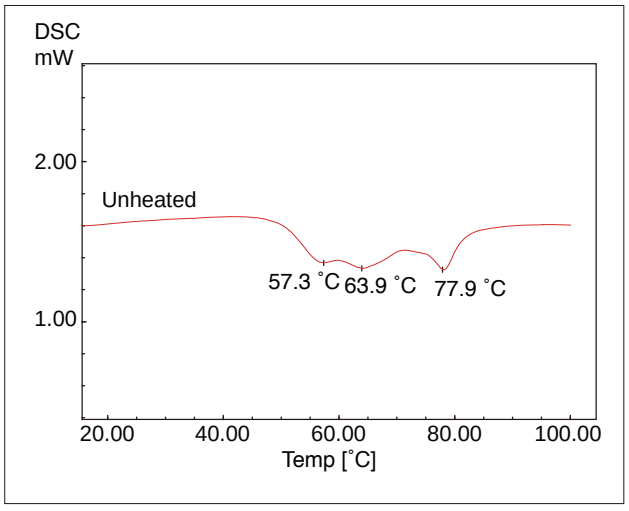


Fig. 8.5.1 DSC Curve of Chicken

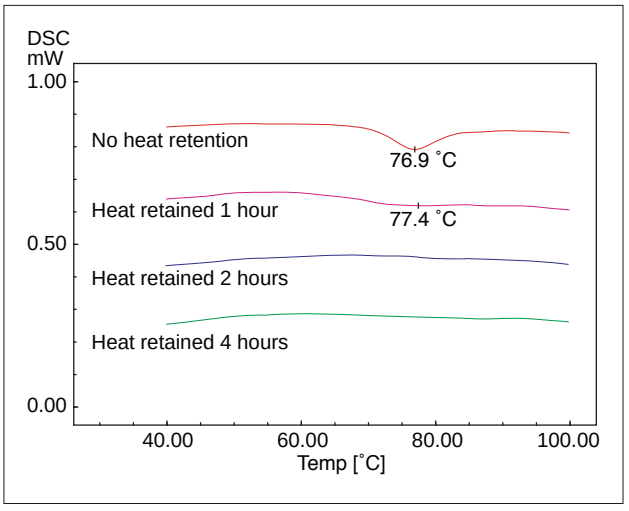


Fig. 8.5.2 DSC Curves of Fried Chicken Subjected to Different Heat Retention Times

8.5 Protein Denaturation and Texture Analysis for Chicken (2) - TM

■Hardness and Elasticity Due to Different Heat Retention Time

Next, the chicken breast fiber was evaluated for hardness by orthogonal shearing, and for elasticity. Table 8.5.1 shows the testing conditions, and Fig. 8.5.4 shows the force and time graph. Fig. 8.5.6 shows the definition of parameters used to indicate texture. Hardness is indicated by the maximum force first applied, and elasticity is indicated as a ratio of the recovery times after applying the load twice. Table 8.5.2 shows the hardness and elasticity calculated based on these test results. It is clear that while the hard-ness rose marginally up to a heat retention time of 1 hour, the specimen suddenly becomes harder after heat retention exceeds 2 hours. This also correlates to the DSC measurement result in which protein denaturation is seen within one hour.

Fig. 8.5.3 Photograph of AGS-X System

Table 8.5.1 Testing Conditions

Testing Instrument	AGS-X
Load Cell	1 kN
Jig	Tooth-shaped push rod
Test Speed	750 mm/min
Clearance	5 mm (shear indentation amount)
Number of Load Cycles	2 cycles
Software	TRAPEZIUMX (Texture)
Sample Dimensions	About 20 × 20 × 10 mm

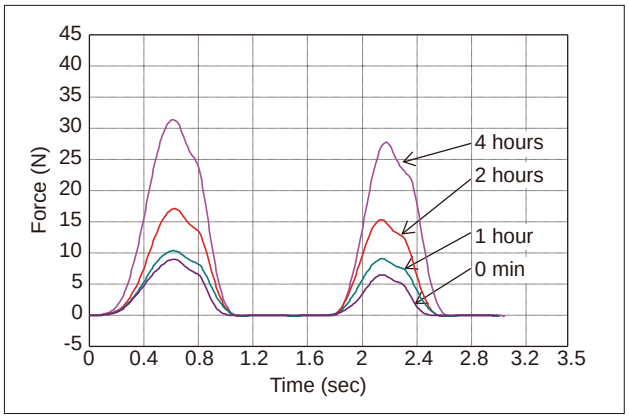


Fig. 8.5.4 Relation between Force and Time

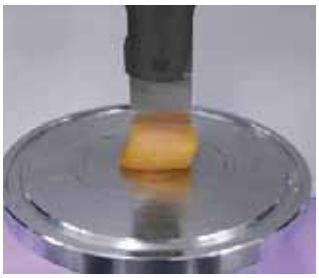


Fig. 8.5.5 Starting Status of Shear Test

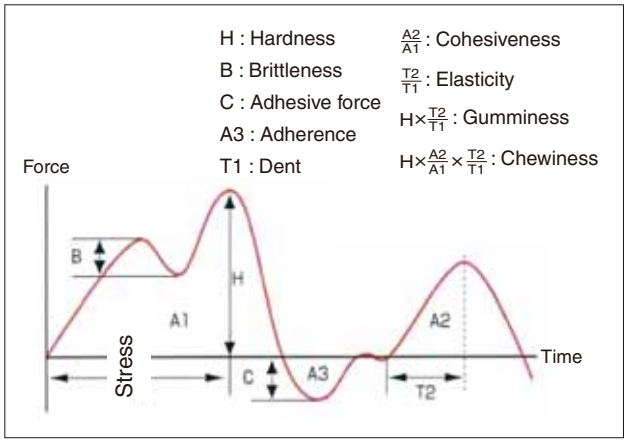


Fig. 8.5.6 Texture-Related Parameters

Table 8.5.2 Test Results

Heat Retention Time	Hardness (N)	Elasticity
0 min	8.87	1.33
1 hour	11.50	2.05
2 hours	16.83	2.98
4 hours	32.77	5.72

8.6 Texture Analysis of "Soumen" Japanese Vermicelli (1) - TM

■Explanation

The evaluation of the mechanical properties of foods, such as strength and hardness, is becoming widely used for the numerical comparison and control of food texture. Here we introduce the tensile test and cutting test of Japanese soumen vermicelli to evaluate the texture. Soumen vermicelli is originated in Nara Prefecture in Japan. It was made by hand by kneading wheat, salt, and water; applying food oil and starch; and then stretching, drying, and maturing. Nowadays, it is generally machine-made. According to JAS (Japan Agricultural Standards) standards, noodles less than 1.3 mm diameter are "soumen," those from 1.3 mm to 1.7 mm diameter are "hiyamugi," and larger noodles are classified as "udon."

■Testing Equipment and Specimens

Two types of commercially available soumen (Sample A, Sample B) were used as specimens for these tests. However, the diameters varied between 0.8 mm and 1.3 mm due to the degree of drying (the state in which it was sold). Therefore, the noodle diameters were measured with Vernier calipers to select specimens of approximately the same diameter. The specimens were added to boiling water and boiled for three minutes and then washed in cold water for ten seconds. Ten specimens each of Sample A and Sample B were tested within five minutes. Testing was performed using a Shimadzu EZ Test tabletop tester (Fig. 8.6.1).

Fig. 8.6.1 EZ Test Tabletop Tester

■Tensile Test

The specimens were grasped in grips (sponge was attached to the grip faces to prevent destruction of the specimens), and the grips were mounted in the tester through universal joints. Tensile test was performed under the following conditions:

1) Force measurement	Load cell (1 N)
2) Extension measurement	Internal extensometer in tester
3) Test speed	50 mm/min

Fig. 8.6.2 and Fig. 8.6.3 show an overview of the grips and a specimen mounted in the tester.

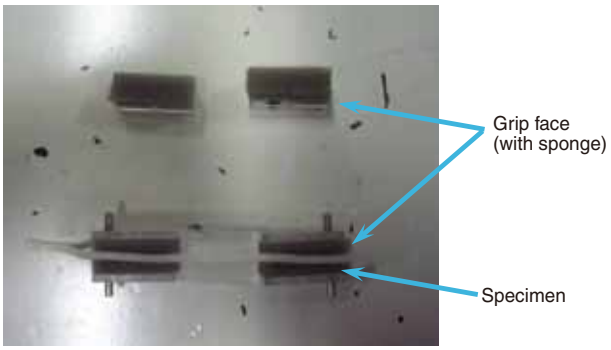


Fig. 8.6.2 Grips for Tensile Test

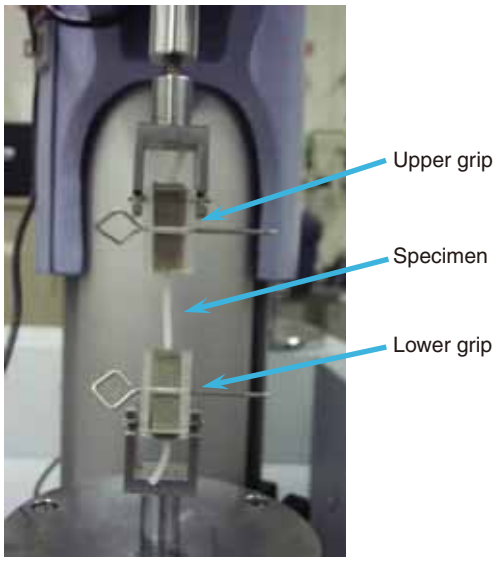


Fig. 8.6.3 Overview of Tensile Test

8.6 Texture Analysis of "Soumen" Japanese Vermicelli (2) - TM

Fig. 8.6.4 shows the tensile test results as force-displacement (extension) curves. (Curves are superimposed for all ten specimens.) The results indicate a maximum force of 164 mN and break displacement of 56 mm for Sample A and 120 mN maximum test force and break displacement of 37.8 mm for Sample B. Sample A exhibits greater extension and strength than Sample B. (All values are averages of ten samples.)

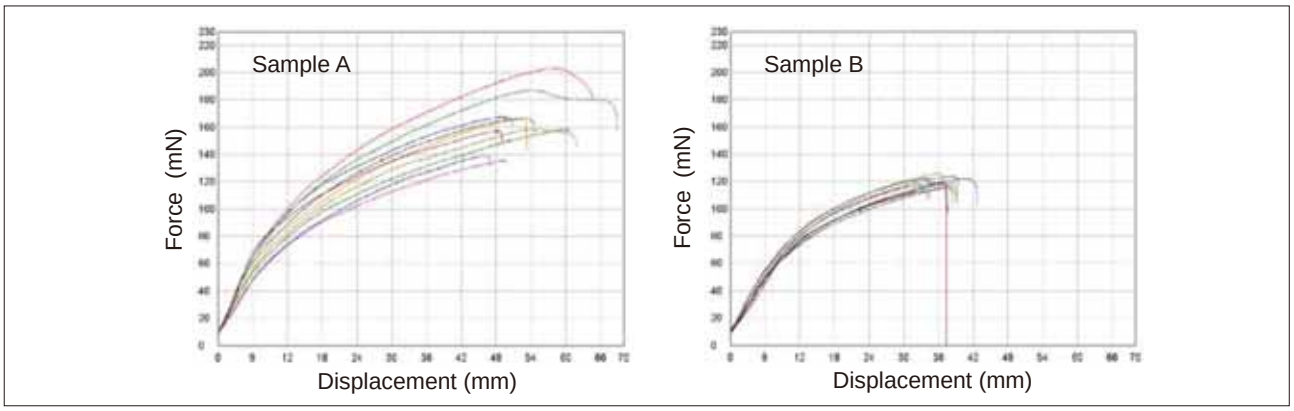


Fig. 8.6.4 Tensile Test Results

■Cutting Test

Cutting test can be performed as an evaluation method that approximates biting through foods. For cutting test, the specimen is placed on the compression plate and the cutting test jig (tooth-shape press: R 0.2 mm knife-edge tip) is pressed down on the sample from above. The test conditions are as follows:

1) Force measurement Load cell (1 N)
2) Indentation measurement Internal extensomete in tester
3) Test speed 5 mm/min

Fig. 8.6.5 shows a specimen mounted in the tester.

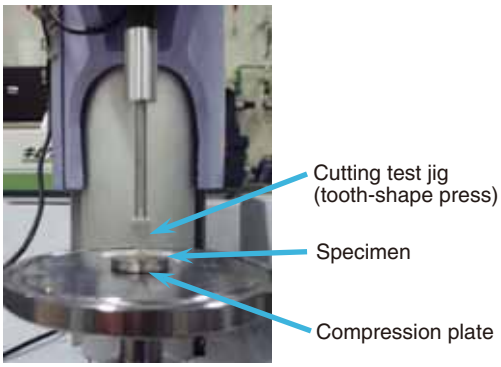


Fig. 8.6.5 Overview of Cutting Test

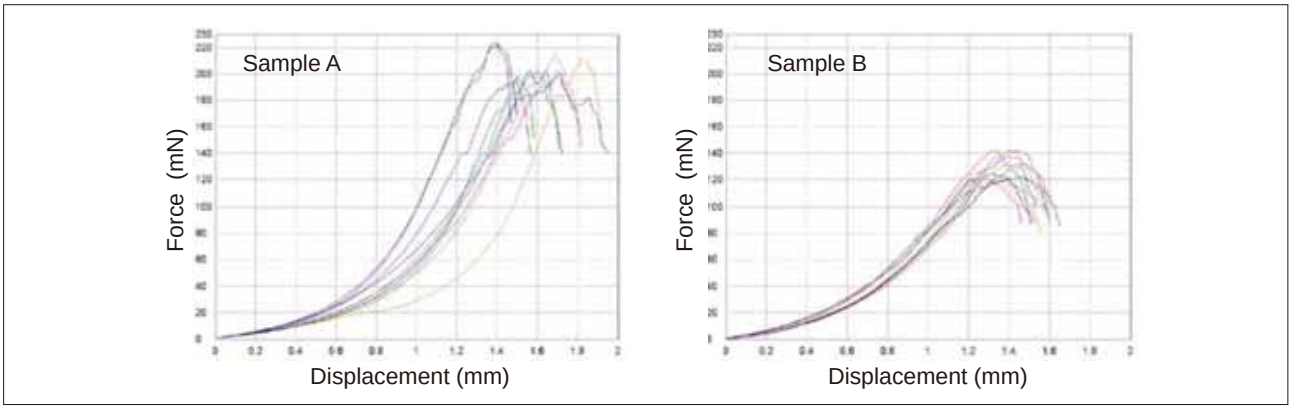


Fig. 8.6.6 Cutting Test Results

Fig. 8.6.6 shows the cutting test results as force-displacement (indentation) curves. (Curves are superimposed for all ten specimens.) The results indicate a maximum force of 207 mN for Sample A and 129 mN maximum test force for Sample B. (All values are averages of ten samples.) The tensile and cutting test results above provide a numerical evaluation that Sample A has a firmer texture than Sample B. The results confirm that the materials tester is effective for quantification for functional testing.

8.7 Measurement of Texture Characteristics of Rice - TM

■Explanation

The EZ Test Shimadzu Texture Analyzer is used for various purposes such as numerical conversion of texture characteristics of foods such as chewiness, firmness, and palatability; quality evaluation based on the change of hardness; and strength evaluation of food packaging. The Analyzer is usable for such a wide range of purposes because a wide variety of jigs can be selectively used according to the type of test being performed. We conducted texture characterization of rice using the Texture Analyzer.

■Samples and Testing Machines

Three types of rice; blended rice, Koshihikari (one of the most popular brands of rice), and rice with barley were used as samples in the measurement. Fig. 8.7.1 shows the appearance of the EZ Test Shimadzu Texture Analyzer used in the measurement. The analyzer has an exceptionally operable, compact frame and is perfect for texture characterization of foods. Table 8.7.1 shows the process to make test pieces and Table 8.7.2 shows the configuration of the system used for the measurement.

Fig. 8.7.1 Appearance of Texture Analyzer

Table 8.7.1 Test Piece Making Process

(1)	Rice was cooked and left as is for one hour.
(2)	Rice was made into 10 g units.
(3)	Each unit of rice was then formed with a mold into a 20 mm high cylindrical form with a diameter of 25 mm.

Table 8.7.2 System Configuration

Main Unit	EZ Test
Load Cell	Capacity 100 N
Jig	50 mm dia. compression plate
Software	TRAPEZIUMX Texture

■Test Conditions

Table 8.7.3 shows test conditions. Fig. 8.7.2 shows how a test piece is placed.

Table 8.7.3 Test Conditions

Test Speed	50 mm/min
Pressing Amount	15 mm
Temperature	28 °C
Humidity	60 %

Fig. 8.7.2 Test Piece Placed

■Test Results

Table 8.7.4 shows "Summary of Test Results (Average Values)" and Fig. 8.7.3 shows a "Force-Time" measurement example.

Table 8.7.4 Summary of Test Results (Average Values)

Sample	Hardness [N]	Cohesion Strength [N]	Cohesiveness [Nmm]
Blended Rice	7.86	0.54	0.967
Koshihikari	16.3	1.09	2.78
Rice with Barley	7.67	0.33	0.603

Hardness : The maximum force loaded when compressed
Cohesion Strength : The maximum force required to pull off the jig after compression
Cohesiveness : A value calculated by multiplying the force required to pull off the jig after compression by the distance

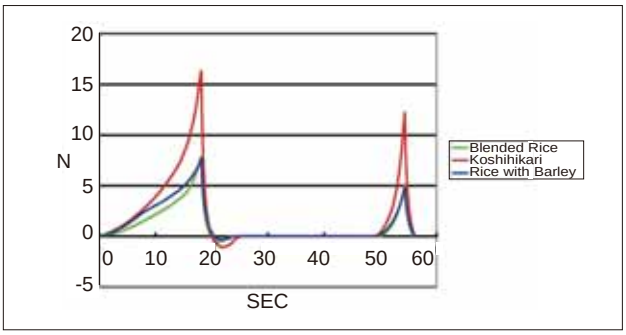


Fig. 8.7.3 Force-Time Graph

■Summary

Koshihikari showed over twice as high values as compared to the other two types of rice for all items; hardness, cohesiveness, and cohesion strength. Cohesiveness is believed to closely indicate the sensation people fell when they actually eat rice. The test result for cohesiveness clearly indicates Koshihikari's characteristics. The use of the EZ Test Texture Analyzer is recommended for evaluating food texture because it provides easier texture characterization as compared to sensory evaluations and is highly operable.

8.8 Measurement of Texture of Pork - TM

■Explanation

Nowadays people like tender meat more than chewy meat. Therefore, various measures are taken to make pork tender, for example by soaking it in water or in protein degrading enzyme solution to quickly achieve an adequate level of tenderness. Two cuts of store-bought Boston pork butt were soaked for 20 hours, one in water and the other in a protein degrading enzyme solution. A penetrating strength test was then performed on them and their textures were converted into numerical values for comparison. Fig. 8.8.1 is the penetrating test force-penetrating depth curve of store-bought Boston pork butt. Fig. 8.8.2 shows how the penetrating strength test was performed.

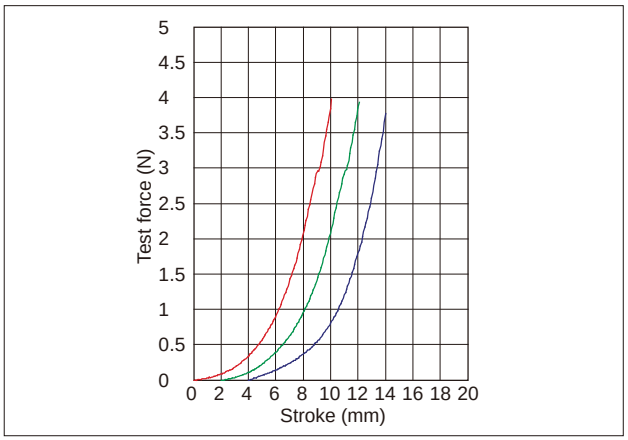


Fig. 8.8.1 Penetrating Test Force-Penetrating Depth Curve on Store-Bought Boston Pork Butt

Fig. 8.8.2 Penetrating Strength Test Being Performed on Pork

The EZ Test Shimadzu Table-Top Universal Tester was used in the test. Fig. 8.8.3 is the penetrating test force-penetrating depth curve of pork soaked in water for 20 hours. Fig. 8.8.4 shows the results of soaking meat in a protein degrading enzyme solution for 20 hours.

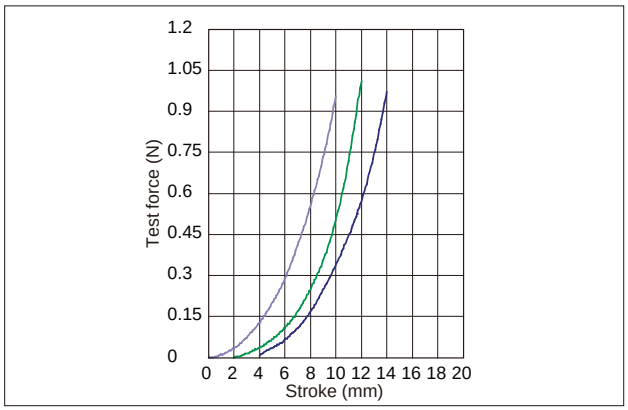


Fig. 8.8.3 Curve of Pork Soaked in Water for 20 Hours

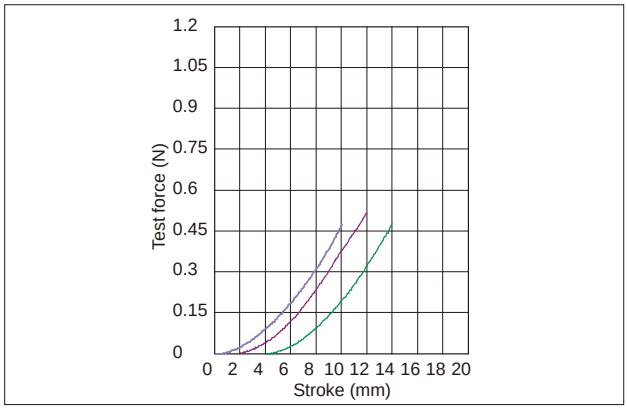


Fig. 8.8.4 Curve of Pork Soaked in Enzyme Solution for 20 Hours

In these tests, the strength was measured at the point where a 5 mm diameter penetration elasticity jig penetrated a 20 mm thick cut of Boston Pork Butt to a depth of 10 mm at a speed of 100 mm/min. When the results shown in Fig. 8.8.3 and Fig. 8.8.4 are compared, the jig went deeper into the pork soaked in enzyme solution for 20 hours as compared to the pork soaked in water for 20 hours when the same test force was used, indicating the former is more tender. When Fig. 8.8.1 and Fig. 8.8.3 are compared, approximately four times the strength is necessary to penetrate store-bought pork to the same depth as pork soaked in water for 20 hours, clearly indicating the difference in hardness. As shown in these figures, the texture of pork was converted into numerical values from the pork penetrating test strength-penetrating depth curve. Table 8.8.1 below shows the maximum penetrating test strength of these three tests.

Table 8.8.1

Sample	Average Value of Maximum Test Force N
Store-Bought Boston Pork Butt	3.90
Boston Pork Butt Soaked in Water for 20 hours	0.98
Boston Pork Butt Soaked in Enzyme Solution for 20 hours	0.49

8.9 Texture Evaluation of Care Food (1) - TM

■Explanation

The development of care food, which is processed to be soft and easy to eat, is steadily progressing to the point that the shape, color and taste are nearly indistinguishable from ordinary food, except that the texture is softened to enable crushing using just the gums and tongue. These commercially available products are a great boon to the elderly and individuals with internal mouth injuries who may have inadequate strength for chewing and drinking, and are helping these disadvantaged groups to better enjoy the pleasures of tasting and eating. Meanwhile, according to the Health Promotion Law in Japan, the Japan Consumer Affairs Agency is authorized to conduct reviews of the suitability of "foods for people with dysphagia" as regulated special purpose foods, and based on these reviews, permit the display of such foods. Here, we conducted measurements of commercially available care food based on the test methods specified for reviewing the suitability of foods for people with dysphagia. We then classified the food according to the specific standard based on the obtained hardness, adhesiveness, and cohesiveness data.

■Testing Equipment and Specimens

The instrument used for this evaluation was the Shimadzu EZ Test Texture Analyzer, with a 5 N test force measurement load cell. The samples used consisted of 3 commercially available care food products (sample names: A - C).

■Test Conditions

An overview of the measurement setup is shown in Fig. 8.9.1. The sample diameter was 40 mm, and the sample was loaded to a height of 15 mm in the 20 mm high sample container. Using a plastic plunger 20 mm in diameter and 8 mm in height, compression testing was conducted twice at a speed of 10 mm/sec with a clearance of 5 mm. The test was conducted using a sample temperature of 20 °C.

Fig. 8.9.1 Overview of Texture Test

■Test results

The results obtained from testing 3 types of food products are shown in the force-time graphs of Fig. 8.9.2 - Fig. 8.9.4.

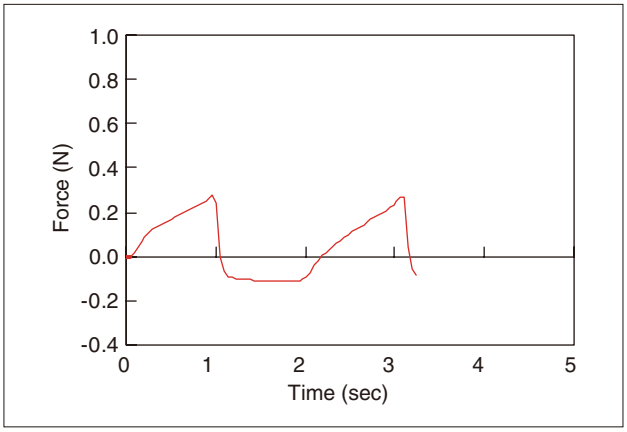


Fig. 8.9.2 Food A (vegetable soup)

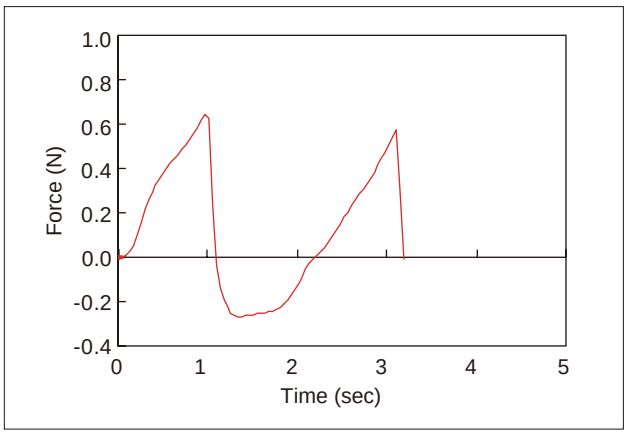


Fig. 8.9.3 Food B (rice gruel)

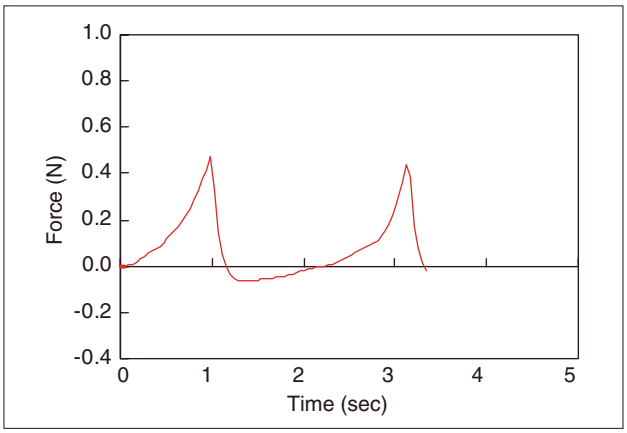


Fig. 8.9.4 Food C (sukiyaki)

8.9 Texture Evaluation of Care Food (2) - TM

The results obtained from the series of measurements are shown in Table 8.9.1.

Table 8.9.1

Sample	Hardness		Adhesiveness		Cohesiveness		Permissible Standard (Overall Judgment)
	Measured Value (N/m^2)	Judgment	Measured Value (J/m^3)	Judgment	Measured Value	Judgment	
Food A	0.84×10^3	Ⅰ, Ⅱ, [Ⅲ]	0.19×10^3	Ⅰ, [Ⅱ], [Ⅲ]	0.82	Ⅰ, [Ⅱ]	Ⅲ
Food B	1.97×10^3	Ⅰ, [Ⅱ], [Ⅲ]	0.41×10^3	Ⅰ, [Ⅱ], [Ⅲ]	0.76	Ⅰ, [Ⅱ]	Ⅲ
Food C	1.88×10^3	Ⅰ, [Ⅱ], [Ⅲ]	0.08×10^3	[Ⅰ], [Ⅱ], [Ⅲ]	0.52	[Ⅰ], [Ⅱ]	Ⅲ

The measured values for hardness, adhesiveness and cohesiveness that are within the range of the permissible standard are indicated with a □ that surrounds the standards in the judgment field. Food A does not satisfy the hardness standards Ⅰ and Ⅱ, so the permissible standard was designated as Ⅲ. In the case of foods B and C, item Ⅰ is not satisfied, so the permissible standard would be presumed to be Ⅱ. However, the permissible standard becomesⅢ in the final evaluation, because food B and food C contain non-homogeneous ingredients. Thus, by defining the numerical value and comparing it with the judgment standards, the judgment can be made as to which permissible standard it corresponds.

[Reference]

(Permissible Standards of Foods for People with Dysphagia Note 1))

Note 1) Regarding permission to display special usage foods (Excerpted from Ministry of Health, Labour and Welfare Food Safety Notification No. 0212001)

Standard*1	Permissible Standard Ⅰ*2	Permissible Standard Ⅱ*3	Permissible Standard Ⅲ*4
Hardness (Resistance during compression at constant rate) (N/m^2)	2.5×10^3 - 1×10^4	1×10^3 - 1.5×10^4	3×10^2 - 2×10^4
Adhesiveness (J/m^3)	4×10^2 or less	1×10^3 or less	1.5×10^3 or less
Cohesiveness	0.2 - 0.6	0.2 - 0.9	-

*1 Within the permissible range of the standard under conditions of either of ambient temperature or the normal temperature at which eating takes place.
*2 Homogeneous items (for example, jellied foods)
*3 Homogeneous items (for example, jellied or smooth foods). However, this excludes foods that satisfy permissible standard Ⅰ.
*4 Includes non-homogeneous foods (for example, rice gruel which is easily clumped or collected, soft paste-like or jellied foods). However, this excludes foods that satisfy permissible standard Ⅰ or permissible standard Ⅱ.

8.10 Particle Size Distribution Measurement of Chocolate (1) - PT

■Explanation

There are several possible parameters for expressing the taste of a piece of chocolate. For example, it can be described in terms of taste characteristics such as sweetness or bitterness, but it is also possible to characterize chocolate in terms of its texture in the mouth, for instance, how readily it melts in the mouth. Included in such parameters is "tongue texture," for which particle size distribution provides a potential index for expressing this parameter in numeric terms. Here we introduce the use of a SALD-2300 laser diffraction particle size analyzer to measure the size distribution of particles in chocolate. Chocolate is a mixture of cocoa mass, which consists of ground cocoa beans mixed with milk, sugar, cocoa butter, and other ingredients. This can be considered as a mixture of various particles in fat. Consequently, the particle size distribution presumably varies depending on the dispersion conditions. In this example, isopropanol at about 45 °C was used as the dispersant.

Fig. 8.10.1 SALD-2300 Laser Diffraction Particle Size Analyzer

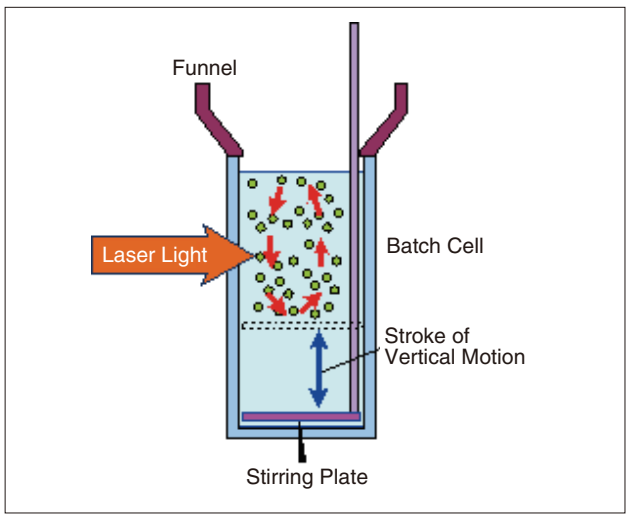


Fig. 8.10.2 Batch Cell

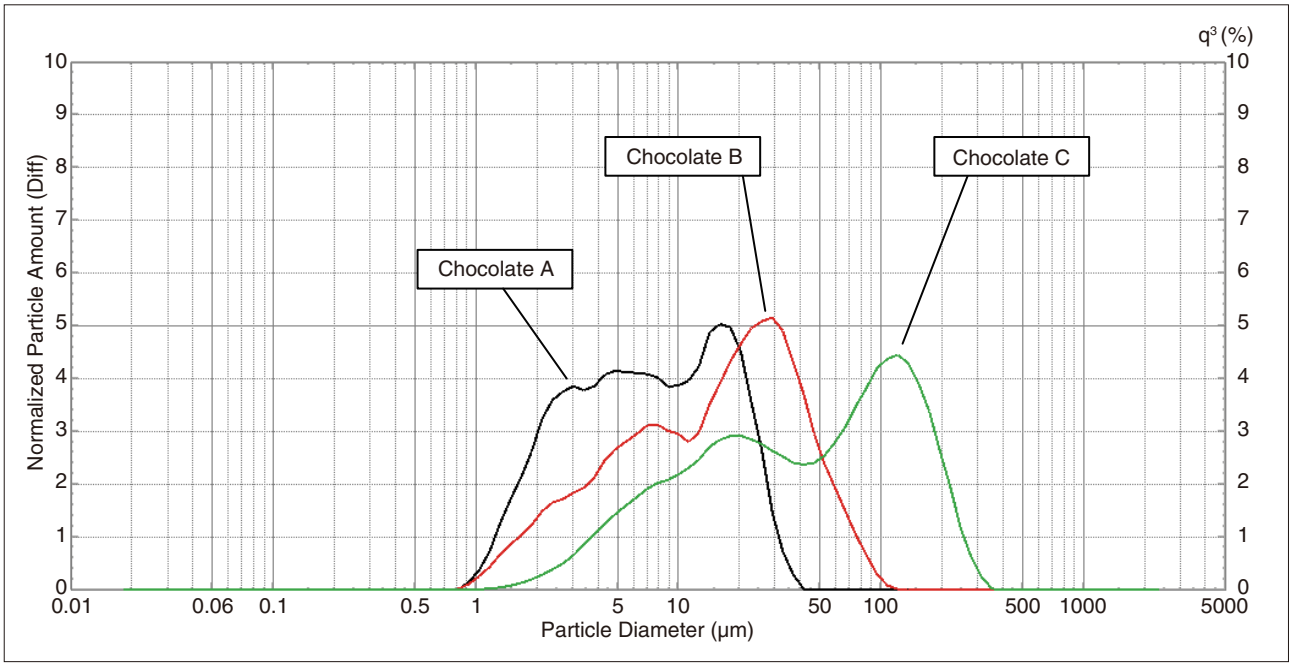
■Analytical Conditions

Dispersant	: Isopropanol (45 °C)
Dispersing Agent	: None
Dispersing Method	: Stirred using a magnetic stirrer
Refractive Index	: 1.70 - 0.05i

8.10 Particle Size Distribution Measurement of Chocolate (2) - PT

■Test Samples and Results

Three kinds of chocolate were prepared as samples. Sample A was milk chocolate, B was milk chocolate from a different manufacturer, and C was chocolate removed from a chocolate chip cookie. Each sample was cut into thin pieces with a box cutter knife and placed in a 50 mL beaker. Then 45 °C isopropanol was added to dissolve (disperse) the samples into a suspension, which was stirred for about 2 minutes with a magnetic stirrer. This suspension was used as the stock solution. Part of this stock solution was sampled and added to a batch cell filled with ambient-temperature isopropanol until the appropriate concentration was achieved. Fig. 8.10.3 shows the particle size distribution results obtained from measurement of the samples as described above. The results indicate that C contains larger particles than B and B contains larger particles than A. When the samples were actually eaten, the mouth texture of Sample A was extremely smooth, B was slightly less smooth than A, and C felt clearly grainy on the tongue. Put simply, the results indicate that an adequately smooth mouth texture can be achieved if all the particles are smaller than 50 µm in diameter. In contrast, chocolate containing particles with diameters larger than 100 µm clearly imparts a grainy sensation. Of course, smoothness on the tongue is not the only factor that determines how good a chocolate product tastes, but this example shows how measuring the particle size distribution provides a measurement scale that can be used for evaluation.



	File Name	Sample ID	Sample #	Absorbance	Refractive Index
1	A	A	bc ipa st2m	0.10	1.60-0.02i
2	B	B	bc ipa st2m	0.08	1.60-0.02i
3	C	C	bc ipa st2m	0.12	1.60-0.02i

	Median D (µm)	Modal D (µm)	Mean V (µm)	Std Dev	10 %D (µm)	50 %D (µm)	90 %D (µm)	0 %D (µm)	0 %D (µm)	0 %D (µm)	0 %D (µm)	0 %D (µm)	0 %D (µm)
1	6.969	14.994	6.771	0.377	2.049	6.969	20.764	0.000	0.000	0.000	0.000	0.000	0.000
2	15.895	30.617	13.265	0.449	2.906	15.895	45.539	0.000	0.000	0.000	0.000	0.000	0.000
3	42.862	127.664	36.861	0.534	6.351	42.862	163.880	0.000	0.000	0.000	0.000	0.000	0.000

Fig. 8.10.3 Particle Size Distributions of Several Kinds of Chocolate

9.1 Identification of Rice Varieties (1) – MCE

■Explanation

Rice is one of Japan's agricultural products for which package labeling of the variety name and production region are obligatory according to the Japanese Agricultural Standard. However, since mislabeling can be problematic, various technologies have been developed to scientifically verify label content. One method that is widely used for assessing the rice variety is to amplify a specific gene region from extracted rice DNA using PCR, and then to analyze the PCR product using an electrophoresis analyzer. Here we introduce a method of conducting multiplex PCR to identify rice varieties using an identification kit capable of distinguishing among 112 commercially available varieties from DNA extracted from polished rice. The electrophoresis patterns of the obtained PCR products were determined using the MCE-202 "MultiNA" Microchip Electrophoresis System.

■Experimental Procedure

Using the rice DNA extraction kit (Takara Bio), DNA was extracted from 20 polished grains each of 5 types of rice (Koshihikari, Akitakomachi, Kinuhikari, Kirara 397, and Hitomebore). Using the extracted DNA as a template, 4 sets of multiplex PCR (sets A, B, C, and D) were conducted using the reagent provided with the rice variety identification kit (Kokken). Each PCR product was analyzed using the MultiNA.

■Reagents/Kits

- Rice DNA Extraction Kit (scaled to 20 grains polished rice) (Takara Bio, 9103)
- Kome Bugyo Series ② Variety Identification Kit (Ver. 2) (Kokken, KK-KB-02-02)
- DNA-1000 Kit (Shimadzu, P/N 292-27911-91)
- SYBR® Gold nucleic acid gel stain (invitrogen, S11494)
- ϕ × 174 DNA / *HaeIII* Markers (Promega, G1761)

■Analytical Conditions for PCR-RFLP Products

Instrument : MCE-202 "MultiNA"
Analysis Mode : DNA-1000 on-chip mode

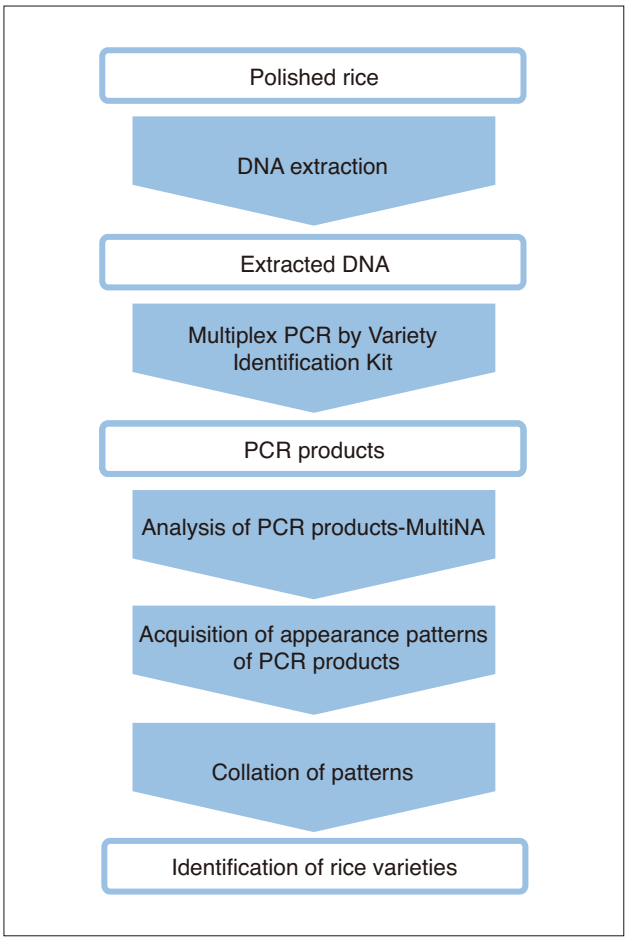


Fig. 9.1.1 Rice Variety Identification Procedure

9.1 Identification of Rice Varieties (2) – MCE

Results

After conducting 4 sets (sets A, B, C, and D) of multiplex PCR using the rice variety identification kit (Kokken) with the templates of DNA extracted from the 5 varieties of rice, the obtained PCR products were analyzed with the MultiNA. The results are shown in Fig. 9.1.2. Five pairs of primers from set A, 4 pairs from set B, 5 pairs from set C, and 5 pairs from set D were used to conduct multiplex PCR. The PCR products obtained with the primer pairs from each set are shown in the respective control lanes. The appearance patterns for 19 PCR products were determined from electrophoresis analysis results of the multiplex PCR of the 4 sets, and the different rice varieties were identified by comparing the obtained patterns with the expected pattern of each variety of rice. The tool that automates the pattern comparing is provided by the kit manufacturer. The all patterns of the PCR products obtained from the analysis results using the MultiNA correspond are consistent with the expected pattern of each variety. MultiNA is the powerful tool for electrophoretic analysis of multiplexed PCR products.

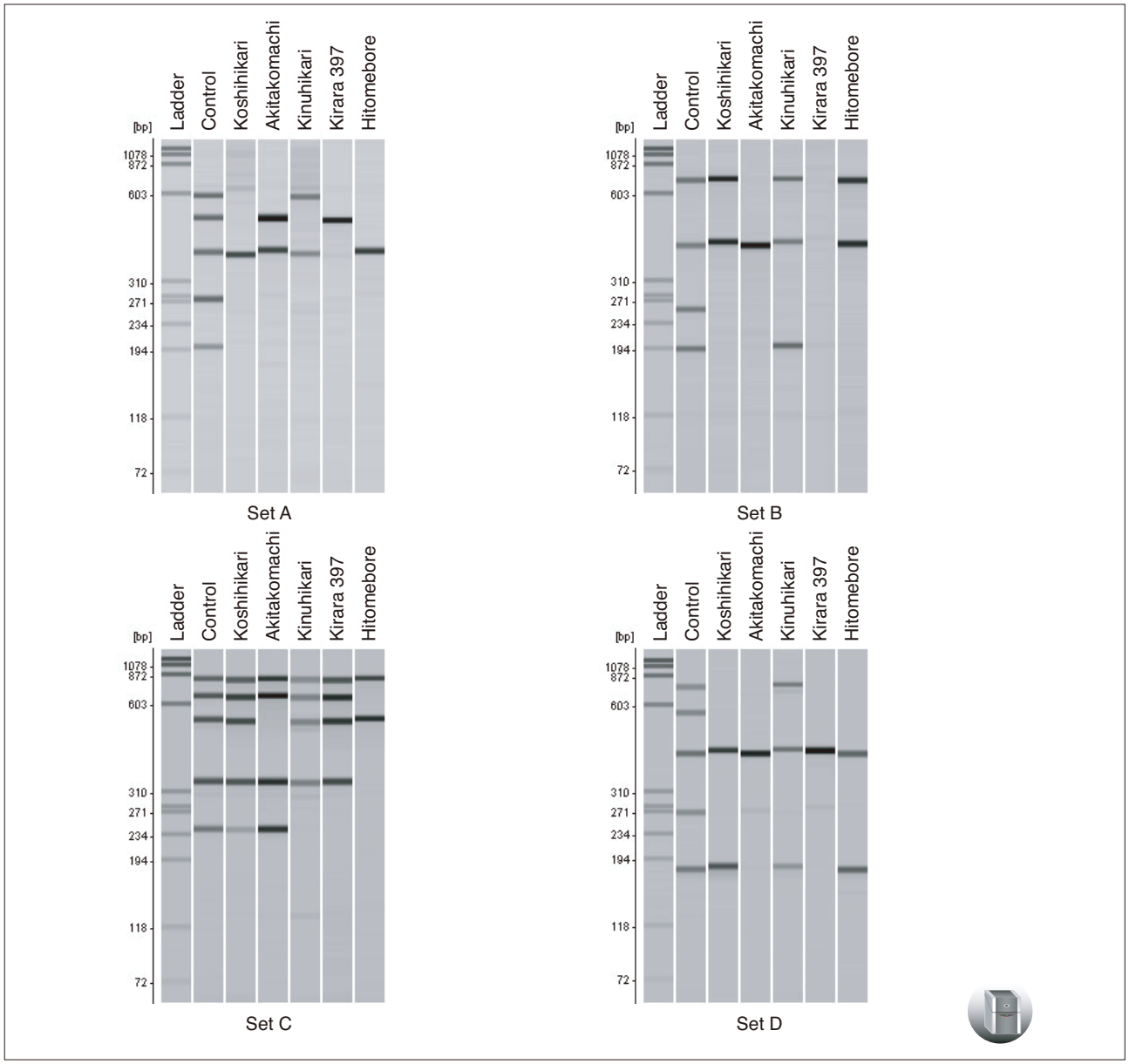


Fig. 9.1.2 Analytical Results of Multiplex PCR of Extracted DNA from Rice Using MultiNA

9.2 Identification of Thunnus (1) – MCE

■Explanation

Consumer concern over food safety has steadily risen in recent years. Responding to this concern, the Japanese Agricultural Standard (Law Concerning Standardization and Proper Labeling of Agricultural and Forestry Products) was revised. The Quality Labeling Standard System was established, requiring the clear and accurate display of food product name, country of origin, etc. It is the responsibility of the manufacturer or the distributor to accurately convey this information as a product selection guideline to consumers. For example, seafood belonging to the tuna species, which is consumed in large quantities by the Japanese, is difficult to distinguish among the various types when presented in the fresh or processed seafood state. Therefore, misidentification, inaccurate labeling and disguise during the distribution process are considered to be social problems, therefore requiring a technique that can quickly, simply and accurately distinguish among product varieties. Here we introduce the procedure for distinguishing the differences among fish stock of Atlantic bluefin tuna (Thunnus thynnus), southern bluefin tuna (T. maccoyii), α and β bigeye tuna (T. obesus), yellowfin tuna (T. albacares), and albacore tuna (T. alalunga) using the PCR-RFLP (Polymerase Chain Reaction -Restriction Fragment Length Polymorphism) method as described in the manual produced by the Food and Agricultural Materials Inspection Center and the National Research Institute of Fisheries Science, Fisheries Research Agency. The MCE-202 "MultiNA" microchip electrophoresis analyzer was used for detection of the separation patterns of the PCR-RFLP products used for distinguishing the differences between these types of fish stocks.

■Experimental Procedure

The DNA extraction and PCR conditions conformed to those presented in the Manual for Distinguishing Among Tuna Fish Stocks* produced by the Food and Agricultural Materials Inspection Center and the National Research Institute of Fisheries Science, Fisheries Research Agency. DNA was extracted from pieces of Atlantic bluefin tuna, southern bluefin tuna, α and β bigeye tuna, yellowfin tuna and albacore tuna. PCR was conducted using the DNA extracted from the various types of tuna as templates. Primers specific to the tuna mitochondrial DNA were used for PCR amplification. The obtained PCR products were processed using restriction enzymes (*Alu* I, *Mse* I, *Tsp*509 I). Electrophoresis of the obtained enzyme digest fragments was conducted using the MultiNA, and the fish varieties were distinguished based on the differences in the fragment patterns.

(*) Manual for Identification of Thunnus Species
Food and Agricultural Materials Inspection Center, National Research Institute of Fisheries Science, Fisheries Research Agency
http://www.famic.go.jp/technical_information/hinpyou/pdf/maguro_manual.pdf (Japanese)

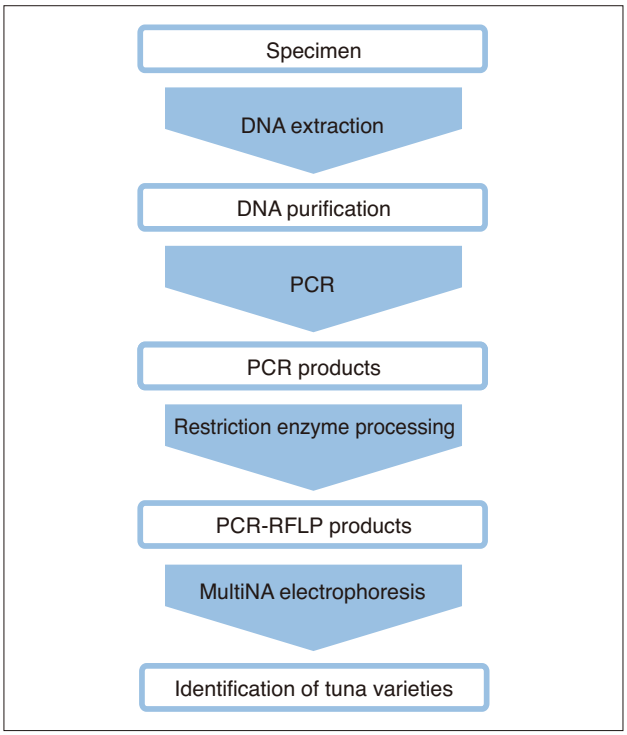


Fig. 9.2.1 Experimental Procedure of Identification of Thunnus Using PCR-RFLP Method

9.2 Identification of Thunnus (2) – MCE

■Reagents/Kits

· DNA-500 Kit (Shimadzu)
· SYBR® Gold nucleic acid gel stain (Invitrogen)
· 25 bp DNA Ladder (Invitrogen)
· DNeasy Blood & Tissue Kit (Qiagen)
· *Alu* I (New England Biolabs Japan) R0137S
· *Mse* I (New England Biolabs Japan) R0525S
· *Tsp* 509 I (New England Biolabs Japan) R0576S

■Analytical Conditions for PCR-RFLP Products

Instrument : MCE-202 "MultiNA"
Analysis Mode : DNA-500 on-chip mode

■Results

Fig. 9.2.2 shows the results of analysis of the PCR-RFLP products from Atlantic bluefin tuna (Thunnus thynnus), southern bluefin tuna (T. maccoyii), α and β bigeye tuna (T. obesus), yellowfin tuna (T. albacares), and albacore tuna (T. alalunga) by MultiNA. The Atlantic bluefin tuna, β bigeye tuna, and albacore tuna show distinctive fragment patterns as a result of *Alu* I restriction enzyme processing, allowing them to be distinguished (*Alu* I processing marked with★ in Fig. 9.2.2). However, the southern bluefin tuna, α bigeye tuna, and yellowfin tuna show the same fragmentation pattern. Therefore, we next performed restriction enzyme processing using *Mse* I. The southern bluefin tuna showed a distinct fragmentation pattern as a result of restriction enzyme processing using *Mse* I, allowing its identification (*Mse* I processing marked with★ in Fig. 9.2.2). As for the remaining α bigeye tuna and yellowfin tuna, 2 types of distinct fragmentation patterns were observed as a result of *Tsp* 509 I restriction enzyme processing, allowing these to be easily distinguished (*Tsp* 509 I processing marked with★ in Fig. 9.2.2). The excellent sensitivity, separation and repeatability of the analysis data obtained with the MultiNA demonstrate that it is a powerful and fully automated tool for determining intra-species genetic variation.

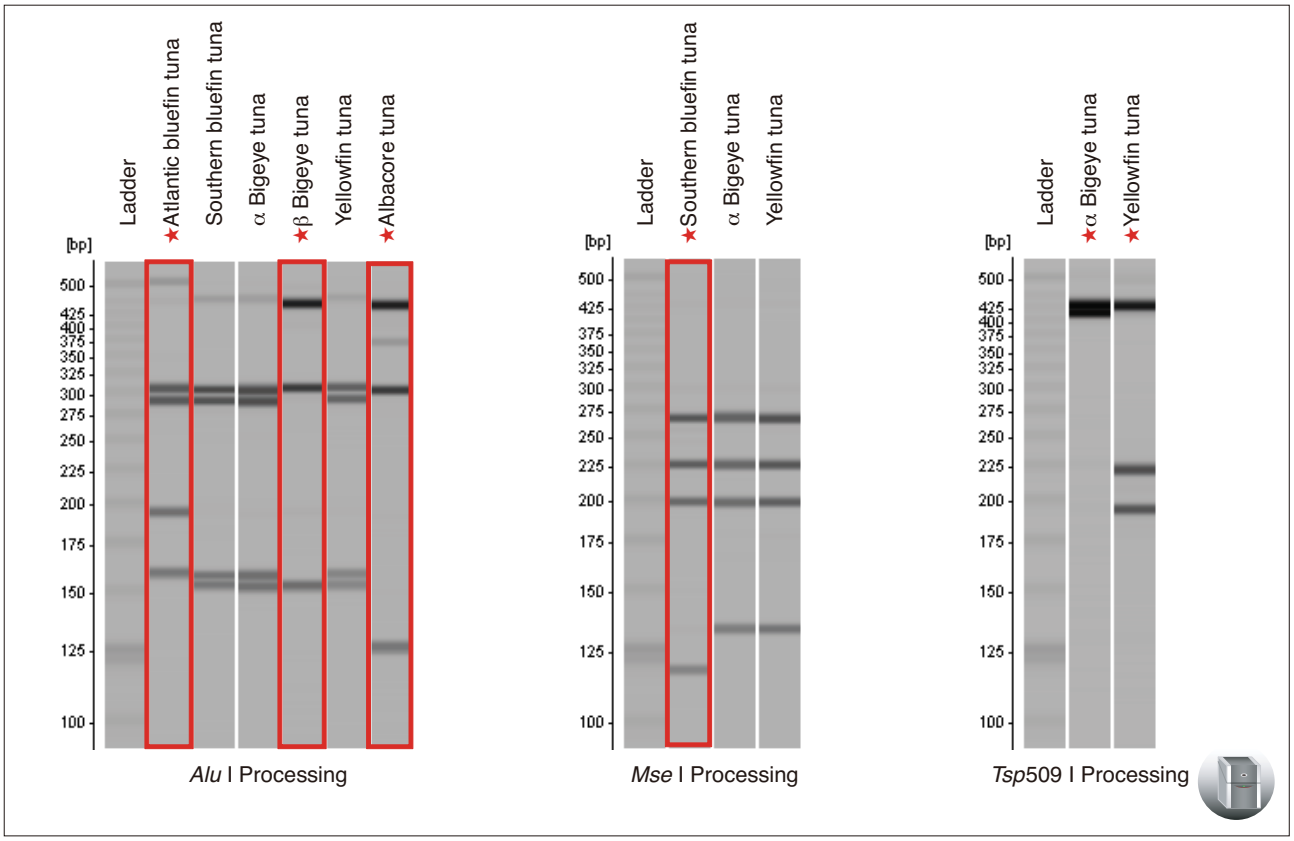


Fig. 9.2.2 Analytical Results for PCR-RFLP Products from Thunnus

9.3 Spectroscopic Measurement and Multivariate Analysis for Classification of Beer Types (1) – UV

■Explanation

Beer is an alcoholic beverage that is quite popular, and consumed in large quantities. Furthermore, the market is now bustling with beverages such as non-alcoholic beer and low-malt beer with beer-like flavors. Beer, low-malt beer, and non-alcoholic beer can all be considered types of beer. A great many varieties of these have been produced and marketed with modifications to the ingredients to adjust such characteristics as alcohol and calorie content. From the standpoint of spectrometric analysis—assuming that the absorption spectra should reflect specific characteristics depending on the types and quantities of ingredients in different beers—we were very interested to see the kinds of differences that would occur upon actual examination. Here, we introduce the results of our investigation into the differences in absorption spectra obtained from measurement of a variety of beers using the UV-3600 Ultraviolet-Visible Near-Infrared Spectrophotometer. Also presented here is our attempt to classify different types of beer using multivariate analysis.

■Samples and Measurement Results

The absorption spectra of 14 types of commercially available beers (4 types of beer, 6 types of low-malt beer, 4 types of non-alcoholic beer) were measured using the UV-3600. Degassing was conducted using ultrasonic irradiation for 3 minutes, and measurement was conducted using a 2 mm optical path length quartz cell and a blank sample consisting of air. The measurement results are shown in Fig. 9.3.1. In addition, expanded spectra of the ultraviolet region (230 - 400 nm) and the near-infrared region (1400 - 1500 nm and 1650 - 1750 nm) are shown in Figs. 9.3.2 through 4, respectively. The large peak in the vicinity of 1450 nm in Fig. 9.3.3 is due mainly to the absorption of water, while the peak in the vicinity of 1695 nm in Fig. 9.3.4 is attributed mainly to ethanol absorption. For reference, the absorption spectra of water and ethanol (99.5 %) are shown in Fig. 9.3.5. It is clear that the peak indicated by the arrow near 1450 nm corresponds to the peaks in Fig. 9.3.3, and that the peak indicated by the arrow near 1695 nm corresponds to the peaks in Fig. 9.3.4. Due to the apparent signal saturation in the ultraviolet region of this data, the absorption spectra were measured in the ultraviolet region (230 - 400 nm) once again after diluting all of the samples 5-fold with distilled water. Those results are shown in Fig. 9.3.6. The peaks that can be seen in the 230 - 300 nm region are believed to be due mainly to absorption of the protein contained in beers.

The alcohol content in the samples is, for beer: 5 %; low-malt beer: 3 - 5.5 %; non-alcoholic beer: 0 %. The protein content per 100 mL is, for beer: 0.2 - 0.4 g; and for low-malt and non-alcoholic beer: 0 - 0.3 g. The alcohol content for each sample is indicated on their product labels as some "value" or "range" within the above-mentioned ranges. The degrees of absorption in the ultraviolet and nearinfrared regions in these data approximately reflect the given content values for protein and alcohol, respectively.

■Analytical Conditions

Instrument	: Shimadzu UV-3600 Ultraviolet-Visible Near-Infrared Spectrophotometer
Measurement Wavelength Range	: 230 nm – 1900 nm
Scan Speed	: Medium
Sampling Pitch	: 1.0 nm
Photometric Value	: Absorbance
Slit Width	: 3 nm
Detector Switching Wavelengths	: 870 nm, 1650 nm

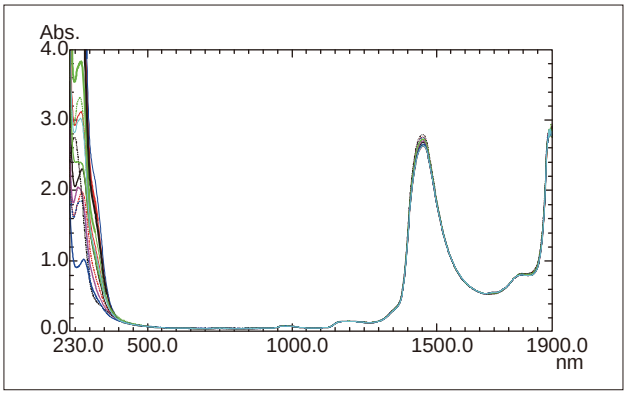


Fig. 9.3.1 Absorption Spectra of Beers, Low-Malt Beers, and Non-Alcoholic Beers (Thick Line: Beers; Thin Line: Low-Malt Beer; Dotted Line: Non-Alcoholic Beers)

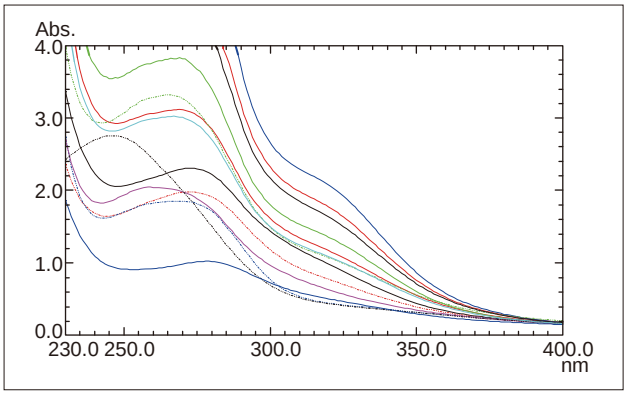


Fig. 9.3.2 Expanded Spectra of Fig. 9.3.1 (230 - 400 nm) (Thick Line: Beers; Thin Line: Low-Malt Beers; Dotted Line: Non-Alcoholic Beers)

9.3 Spectroscopic Measurement and Multivariate Analysis for Classification of Beer Types (2) – UV

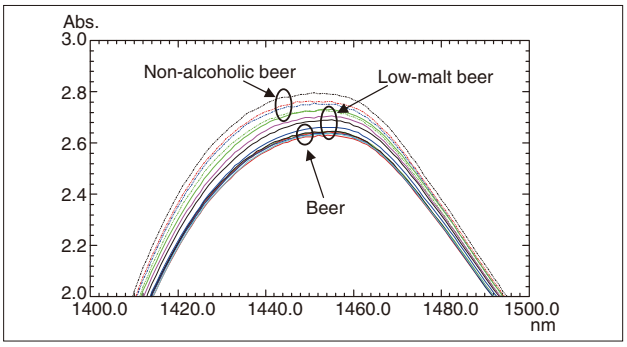


Fig. 9.3.3 Expanded Spectra of Fig. 9.3.1 (1400 - 1500 nm) (Thick Line: Beers; Thin Line: Low-Malt Beers; Dotted Line: Non-Alcoholic Beers)

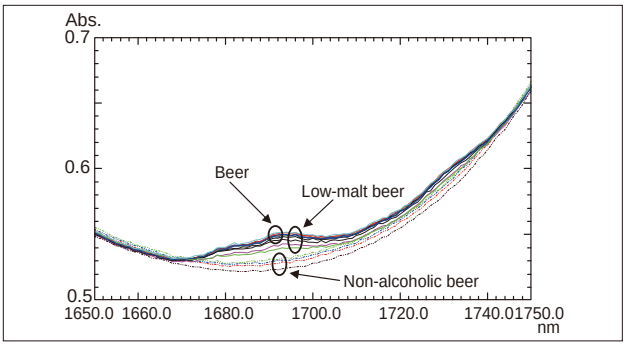


Fig. 9.3.4 Expanded Spectra of Fig. 9.3.1 (1650 - 1750 nm) (Thick Line: Beers; Thin Line: Low-Malt Beers; Dotted Line: Non-Alcoholic Beers)

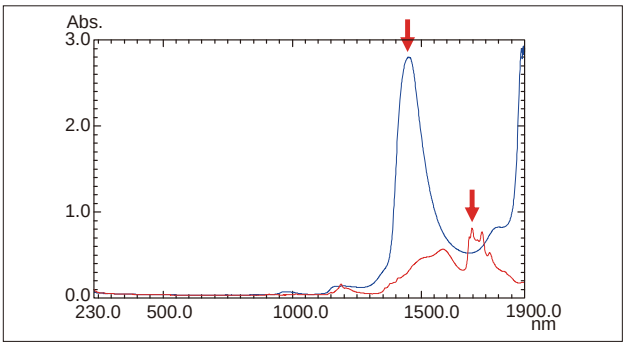


Fig. 9.3.5 Absorption Spectra of Water and Ethanol (Blue Line: Water; Red Line: Ethanol)

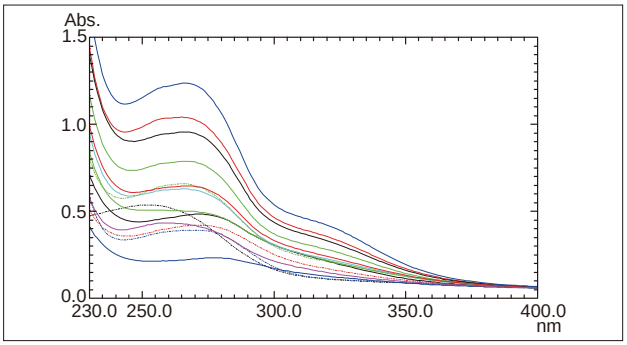


Fig. 9.3.6 Absorption Spectra of Samples Diluted 1:5 (Thick Line: Beers; Thin Line: Low-Malt Beers; Dotted Line: Non-Alcoholic Beers)

■Classification of Beer Types by Multivariate Analysis

We attempted to classify the beers using multivariate analysis. Using the 5-fold dilution absorbance data (230 - 400 nm) and the undiluted sample absorbance data (401 - 1870 nm), we conducted principal component analysis (PCA)[1]. The obtained score plot[2] is shown in Fig. 9.3.7. "A" corresponds to beer, "B" to low-malt beer, and "C" to non-alcoholic beer. The samples of A, B, and C are clearly grouped accordingly. The closer the points are to each other on the score plot, the greater the corresponding samples should resemble one another. Accordingly, A1 and A3, and C1 and C2 should be similar to each other, and in fact, as can be seen from their respective ultraviolet spectra shown in Fig. 9.3.8, they are similar. Looking at the loading plot[3] shown in Fig. 9.3.9 reveals the characteristics of the various groups. As shown in Fig. 9.3.9, loading vector components[3] corresponding to the data components of the ultraviolet region are plotted to the right (or upper right) of the center. This indicates that the further to the right a sample is plotted in Fig. 9.3.7, the greater its ultraviolet absorption will be. The beer samples A1 - A4, which are actually distributed in that direction, display high ultraviolet absorbance as shown in Fig. 9.3.6. Also, in the loading plot of Fig. 9.3.9 there are many loading vector components plotted in the upper left quadrant from 1400 - 1480 nm, which corresponds to the absorption of water. This means that the further to the upper left a sample is plotted on the score plot, the closer that sample is to pure water, and the lower the alcohol content. The non-alcoholic beer samples C1 - C4, which are actually distributed in that direction, display high absorbance in the vicinity of 1450 nm, the wavelength associated with water absorption, as shown in Fig. 9.3.3. From the above, it is clear that the further to the right the samples are plotted on the score plot, the greater their ultrav iolet absorbance values will be. Correspondingly, the further to the upper left the samples are plotted, the lower their alcohol content will be. Put another way, the further to the right a sample is plotted on the score plot, the greater the amount of organic matter (e.g., protein) it will contain; the further to the upper left a sample is plotted, the lower its alcohol content will be. As for the low-malt beers B1 - B6, their positions in the lower left region are probably due to the fact that their absorbance values are not that high in the ultraviolet region (comparable to those of non-alcoholic beer), while several of them have alcohol content comparable to that of beer.

9.3 Spectroscopic Measurement and Multivariate Analysis for Classification of Beer Types (3) – UV

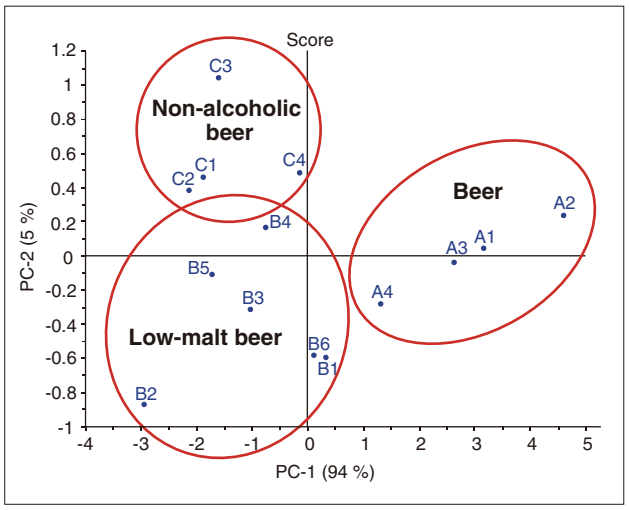


Fig. 9.3.7 Score Plot

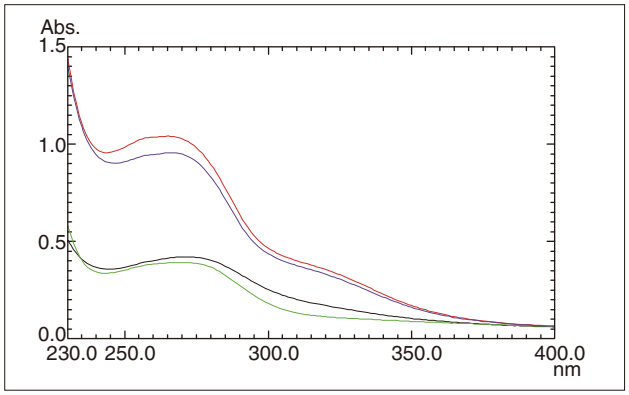


Fig. 9.3.8 Absorption Spectra of A1 and A3, C1 and C2 (Red Line: A1; Blue Line: A3; Black Line: C1; Green Line: C2)

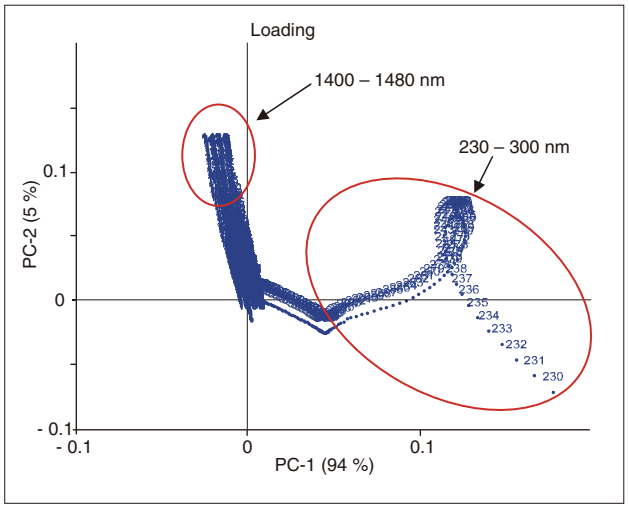


Fig. 9.3.9 Loading Plot

Summary

We were able to confirm the possibility in this investigation of determining the differences in alcohol and protein content in beers by examining their absorption spectra. Further, by applying multivariate analysis to the acquired measurement data, we were able to classify the groups according to the type of beer, and thereby gain an understanding of their characteristics. Comparative investigation of many products is required in the research and development of food products, but an understanding of the degree of similarity among samples is possible using principal component analysis (PCA). The results obtained in this study suggest that a combination of spectral analysis and multivariate analysis can be effective in the development of food products, including beer.

1) The Unscrambler®, a multivariate analysis software application, was used for conducting analysis. The Unscrambler is a trademark or registered trademark of CAMO.
   Regarding the present analysis, principal component analysis was conducted using mean centering of the acquired data.
2) A score plot involves the projection of each sample point expressed in multidimensional space on two loading vectors, expressed as a two-dimensional graph.
   For a description of "loading vector," refer to the following Note 3.
3) A loading plot refers to the plotting via two-dimensional coordinates of substances corresponding to the loading vectors of the first principal component and second principal component (or another combination of principal components). Here, the loading vector refers to the vector obtained by performing eigenvalue calculation for data matrix.

9.4 Detection of Allergenic Substances (1) – MCE

■Explanation

Japan is the world's earliest adopter of a display system for foods containing allergens. In April, 2001 it become mandatory that food labels include likely allergens, and in June, 2008, two additional food articles, prawn and crab, were added to that list of likely allergens. The dissemination of allergen-related information to the consumer using this label display is meant to help avert the possibility of allergic responses and harm to health beforehand. Therefore, if specific substances are included or mixed in among the other ingredients, even at ultra small quantities, the label is required to inform the consumer to that fact. Among the allergenic substances required to be mentioned on the label, those that can be detected by qualitative PCR are wheat, buckwheat, peanuts, prawn and crab. Here we conducted amplification of allergen-related genes by PCR using extracted DNA as the template, according the method specified by The Japanese Ministry of Health, Labour and Welfare (Ministry of Health, Labour and Welfare Notification "Regarding the testing method for foods containing allergenic substances," No. 0724, Publication No. 1 issued by the Dept. of Food Safety, July 24, 2009). Here we introduce the detection of these substances using the MCE-202 MultiNA Microchip Electrophoresis System for DNA/RNA analysis.

■Experimental Procedure

The DNA extraction and PCR were conducted according to the method and conditions specified in the above-mentioned Ministry of Health, Labour and Welfare Notification. We extracted the DNA from food products containing the wheat, buckwheat, peanuts, prawn and crab, respectively. Ion-exchange resins were used for carrying the extractions. The DNA purity verification and quantitation were conducted using the Shimadzu BioSpec-nano Life Sciences Spectrophotometer.

■Reagents/Kits

· DNA-500 Kit
(Shimadzu) P/N: 292-27910-91
· SYBR® Gold nucleic acid gel stain
(Invitrogen) S-11494
· 25 bp DNA Ladder
(Invitrogen) 10597-011
· QIAGEN Genomic-tip 20/G
(QIAGEN) 10223

■Analytical Conditions for PCR Products

Instrument : MCE-202 "MultiNA"
Analysis Mode : DNA-500 on-chip mode

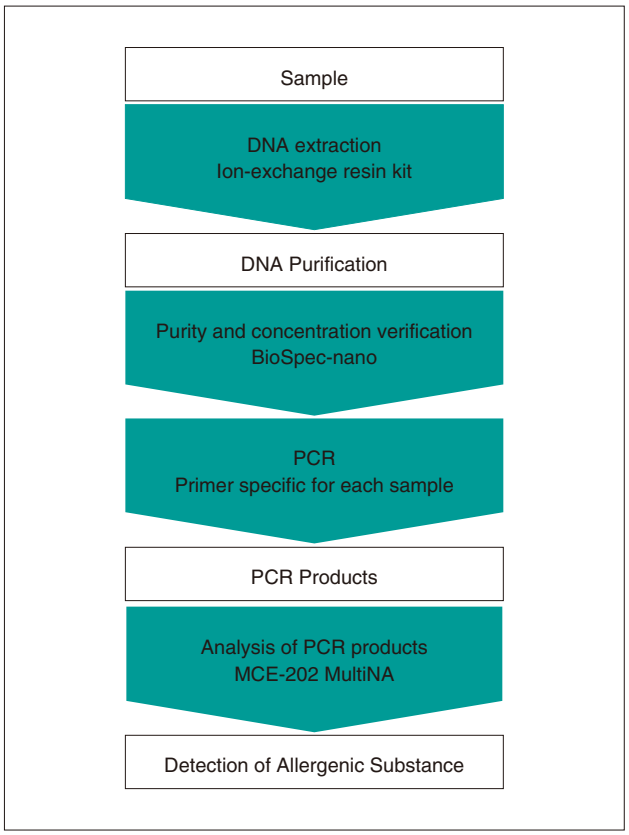


Fig. 9.4.1 Experimental Procedure for Detection of Allergenic Substances

9.4 Detection of Allergenic Substances (2) – MCE

■Results

The results of analysis of the PCR amplification products of DNA derived from wheat, buckwheat, peanuts, prawn and crab, respectively, using the MultiNA are shown in Fig. 9.4.2. The PCR amplification products derived from the wheat, buckwheat, peanuts, prawn and crab substances were all clearly detected using the MultiNA. (The estimated sizes shown in the figure were obtained in this experiment. The sizes of PCR-amplified DNA indicated in The Ministry of Health, Labour and Welfare Notification are wheat: 141 bp, buckwheat: 127 bp, peanuts: 95 bp, prawn: 187 bp, and crab: 62 bp.) The results of analysis by agarose gel electrophoresis of the same samples as those analyzed with the MultiNA are shown in [Reference]. The sizes of the PCR amplification products are imprecise due to lack of objectivity in interpreting the gel electrophoresis results. However, the results obtained using the MultiNA consist of an electropherogram in addition to a gel image, ensuring a high level of accuracy. Particularly noteworthy is that the amplification products of wheat and buckwheat are very near each other. Compared to agarose gel electrophoresis, the MultiNA's excellent resolution and sensitivity allow these to be clearly detected.

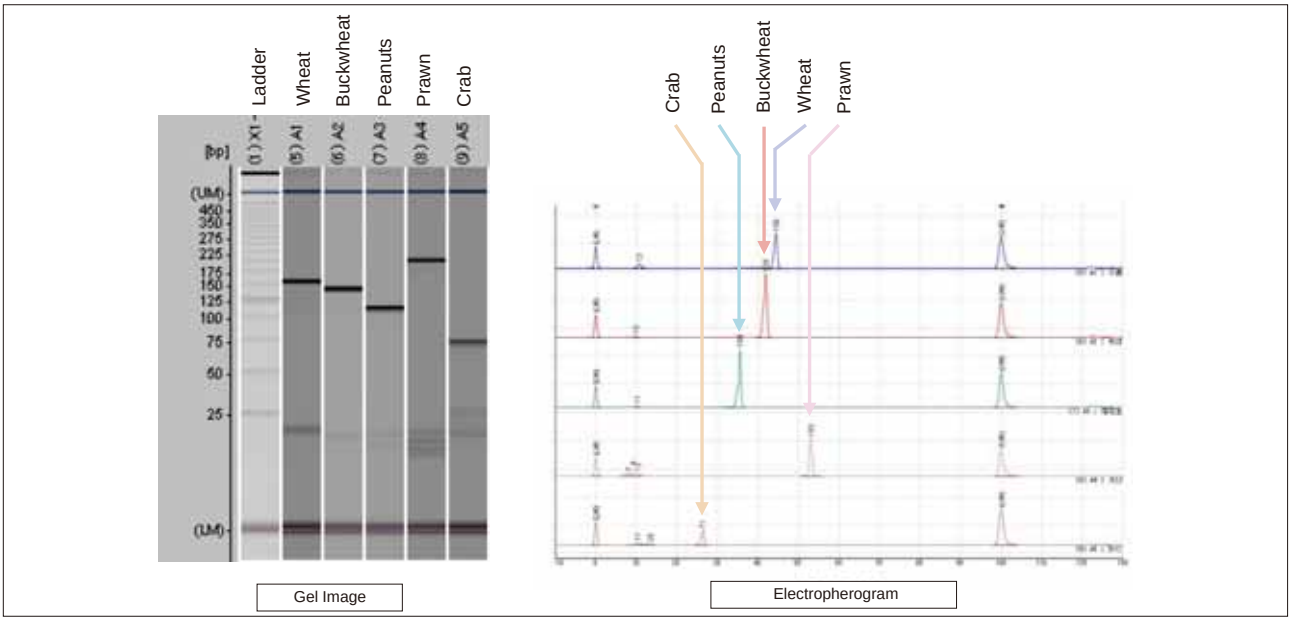
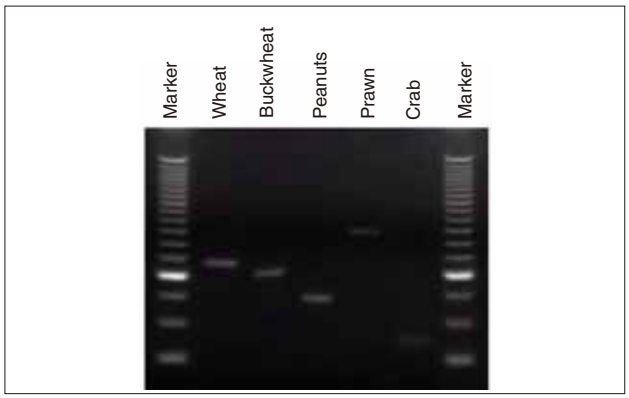


Fig. 9.4.2 Analytical Results for PCR Products from Allergenic Substances



[Reference]
Agarose Gel Electrophoresis of PCR Products from Allergenic Substances

9.5 Detection of Food Poisoning-Related Genes (1) – MCE

■Explanation

In recent years, genetic detection methods have become widely adopted for the identification of the causative agents of food poisoning, allergies, and infectious diseases such as influenza. The most widespread conventional gene-level detection method involves amplification of specific genes using PCR (Polymerase Chain Reaction), followed by detection of the amplification products and sizes using electrophoresis. The conventional agarose gel electrophoresis technique is a labor-intensive series of processes from preparation of the gel to obtaining results. Furthermore, size measurement involves visual comparison with the sizes of known bands, which often leads to variation in results due to the reliance on individual objectivity. Here we introduce the analysis of genes related to food poisoning using the MCE-202 MultiNA Microchip Electrophoresis System for DNA/RNA Analysis. This system offers high-speed, automated analysis, higher detection sensitivity than agarose gel electrophoresis, and automatically calculated size estimation using pretreatment and electrophoretic parallel processing.

■Food Poisoning-Related Genes

Here, the 10 types of genes related to food poisoning shown in Table 9.5.1 were selected as targets.

Table 9.5.1 Food Poisoning-Related Genes

Thermostable direct hemolysin-related hemolysin (*trh* 1&2) gene of *Vibro parahaemolyticus*
Staphylococcus aureus enterotoxin A gene
Staphylococcus aureus toxic shock syndrome toxin-1 gene
*inv*A gene of *Salmonella* sp.
LT gene of enterotoxigenic *E. coli*
STh gene of enterotoxigenic *E. coli*
STp gene of enterotoxigenic *E. coli*
VT1 genes of enterohemorrhagic *E. coli*
VT2 genes of enterohemorrhagic *E. coli*
VT genes of enterohemorrhagic *E. coli*

■Analytical Conditions

(For PCR)
Reagent Kit : Ampdirect® Plus Enzyme Set
Shimadzu P/N: 241-08890-92

(For MultiNA)
Instrument : MCE-202 "MultiNA"
Analysis Mode : DNA-500 on-chip mode
Reagent Kits : DNA-500 Kit
Shimadzu P/N: 292-27910-91
SYBR® Gold nucleic acid gel stain
Invitrogen S-11494
25 bp DNA Ladder
Invitrogen 10597-011

■Experimental Procedure

The samples consisted of DNA extracted from the respective strains, and refined.
PCR was conducted using Shimadzu's Ampdirect® Plus gene amplification reagents, and the obtained PCR amplification products were analyzed using the MultiNA (Fig. 9.5.1).

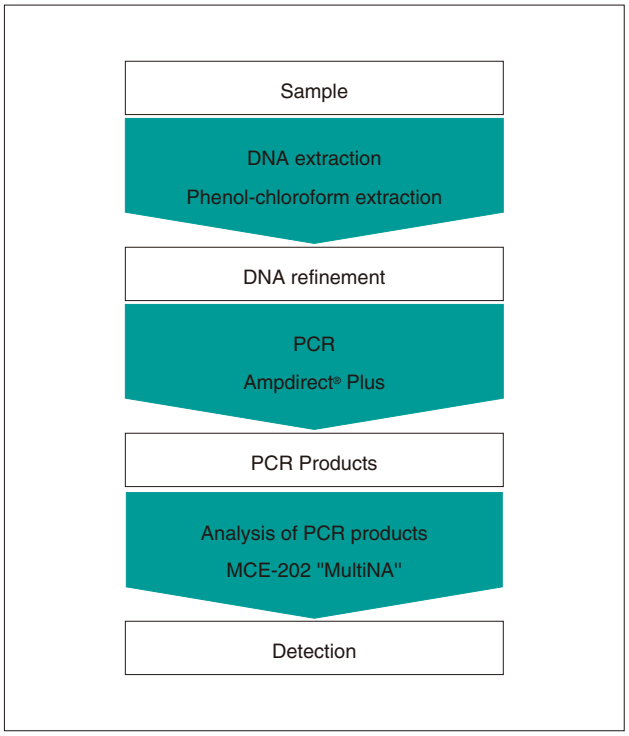


Fig. 9.5.1 Experimental Procedure of Food Poisoning-Related Genes

9.5 Detection of Food Poisoning-Related Genes (2) – MCE

■Detection of Food Poisoning-Related Genes

The results of analysis of the target region for the 10 types of genes related to food poisoning based on the procedure of Fig. 9.5.1 are shown in Fig. 9.5.2. All of the food poisoning-related genes and the targeted regions were detected. The results of analysis using the MCE-202 MultiNA were obtained as electrophoretic images and electropherograms. The estimated size and concentration values for the amplification products were calculated from the calibration curve for the standard sample (ladder) and expressed as numeric values, allowing simple and reliable evaluation of the target amplification products.

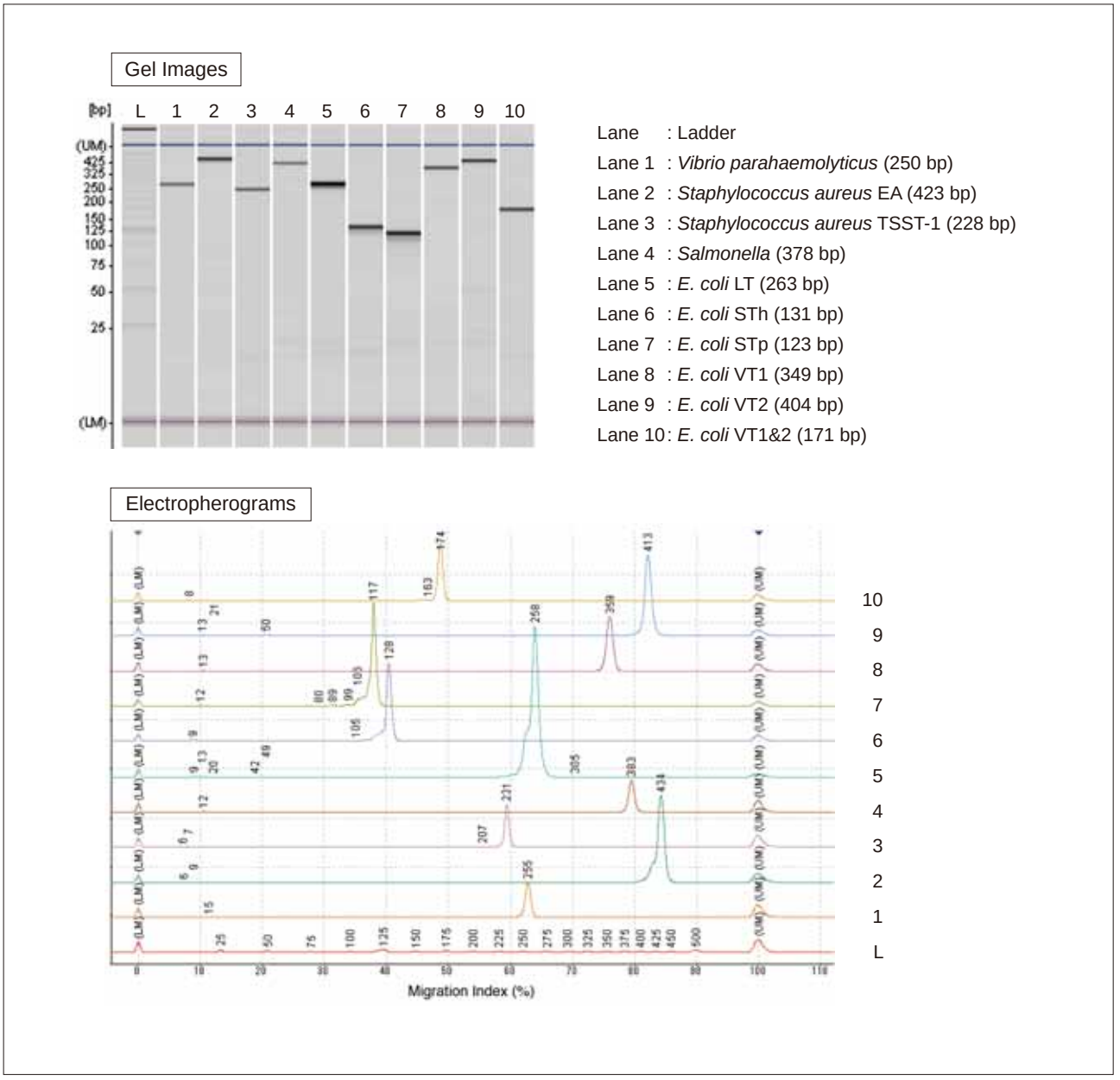


Fig. 9.5.2 Analytical Results of Food Poisoning-Related Genes

9.6 Qualitative Analysis of Genetically Modified Corn (1) – MCE

■Explanation

The "JAS (Japanese Agricultural Standard) Analysis Inspection Handbook, Manual for Inspection and Analysis of Genetically Modified Foods," including standard methods for genetically modified agricultural products and processed goods, is open to the public on the web site of the Ministry of Agriculture, Forestry and Fisheries and the Food and Agricultural Materials Inspection Center, an independent agency.(*1) Inspections for monitoring food labeling of genetically modified agricultural products are conducted by the independent Food and Agricultural Materials Inspection Center based on the JAS Analysis Inspection Handbook. In addition, the handbook is quoted in several places in the "Inspection Method for Food Produced by Recombinant DNA Technology" based on the Ministry of Health, Labour and Welfare notification, attesting to the fact that this analysis method has become one of the standards for inspection of genetically modified agricultural products in Japan. Furthermore, not limited to compliance through government control, the analysis method is widely used as a voluntary inspection system throughout enterprises involved in food processing and distribution to ensure observance of the food labeling system. Here we introduce an example of detection through qualitative inspection of genetically modified corn as specified in the JAS analysis inspection handbook, using the MCE-202 "MultiNA" microchip electrophoresis DNA/RNA analyzer.

■Experimental Procedure

Three certified standards consisting of genetically modified corn powder (MON 810 maize line) were used (Table 9.6.1). The conditions used from DNA extraction to PCR were in accordance with the "JAS Analysis Inspection Handbook, Manual for Inspection and Analysis of Genetically Modified Foods, Basic Operation Volume."(*1) The DNA was extracted from 1 g taken from each of the samples using the Qiagen DNeasy Plant Maxi kit. The extracted DNA concentration was measured, and based on this measurement, the solution was diluted to 10 ng/μL for use as a PCR template. In PCR, 2 types of primer pairs were used for detection of the endogenous corn gene SSIIb, and for detection of the recombinant MON 810 maize line. PCR was conducted from 3 points of each sample extract. In addition, PCR was also conducted on a positive control (standard plasmid DNA for corn added as a template) and negative controls (one without template DNA, the other without primer). The obtained PCR products were analyzed using the MultiNA.

Table 9.6.1 Genetically Modified Corn Samples

Lepidoptera Resistant Maize		
IRMM*2	CRM*3	MON 810 Maize Line 0 % (GVO standard ERM-BF413a)
IRMM	CRM	MON 810 Maize Line 1 % (GVO standard ERM-BF413d)
IRMM	CRM	MON 810 Maize Line 5 % (GVO standard ERM-BF413f)

(*1) Ministry of Agriculture, Forestry and Fisheries Food Label System Q&A and guideline, etc.
http://www.maff.go.jp/j/jas/hyoji/qa.html
Food and Agricultural Materials Inspection Center "Manual for Inspection and Analysis of Genetically Modified Foods" <Revised 2nd Edition>
http://www.famic.go.jp/technical_information/jashandbook/index.html
(*2) Institute for Reference Materials and Measurements (IRMM)
(*3) Certified Reference Materials (CRM)

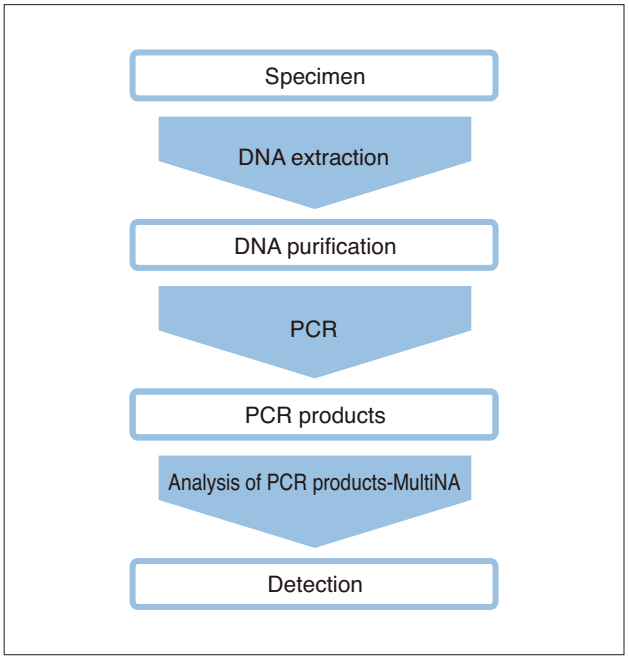


Fig. 9.6.1 Experimental Procedure for Genetically Modified Corn

9.6 Qualitative Analysis of Genetically Modified Corn (2) – MCE

■Reagents/Kits

· DNA-500 Kit
(Shimadzu)
· SYBR® Gold nucleic acid gel stain
(Invitrogen)
· 25 bp DNA Ladder
(Invitrogen)
· DNeasy Plant Maxi Kit
(Qiagen)

■Analytical Conditions for PCR Products

Instrument : MCE-202 "MultiNA"
Analysis Mode : DNA-500 on-chip mode

■Results

From Fig. 9.6.2, it is clear that the endogenous corn gene (SSIIb: 114 bp) was detected in all of the samples. This confirms that the DNA extraction and PCR were achieved without problem. On the other hand, only in the samples containing the MON 810 maize line could the band (M 810: 113 bp) be identified. In addition, the presence/absence of assumed DNA amplification products was confirmed in the positive and negative controls, indicating that the results were accurate. Both an electropherogram and a gel image were obtained in the analysis results using the MultiNA. Therefore, the amplification products of interest were reliably and easily identified by the presence/absence of peaks and their sizes verified two-dimensionally.

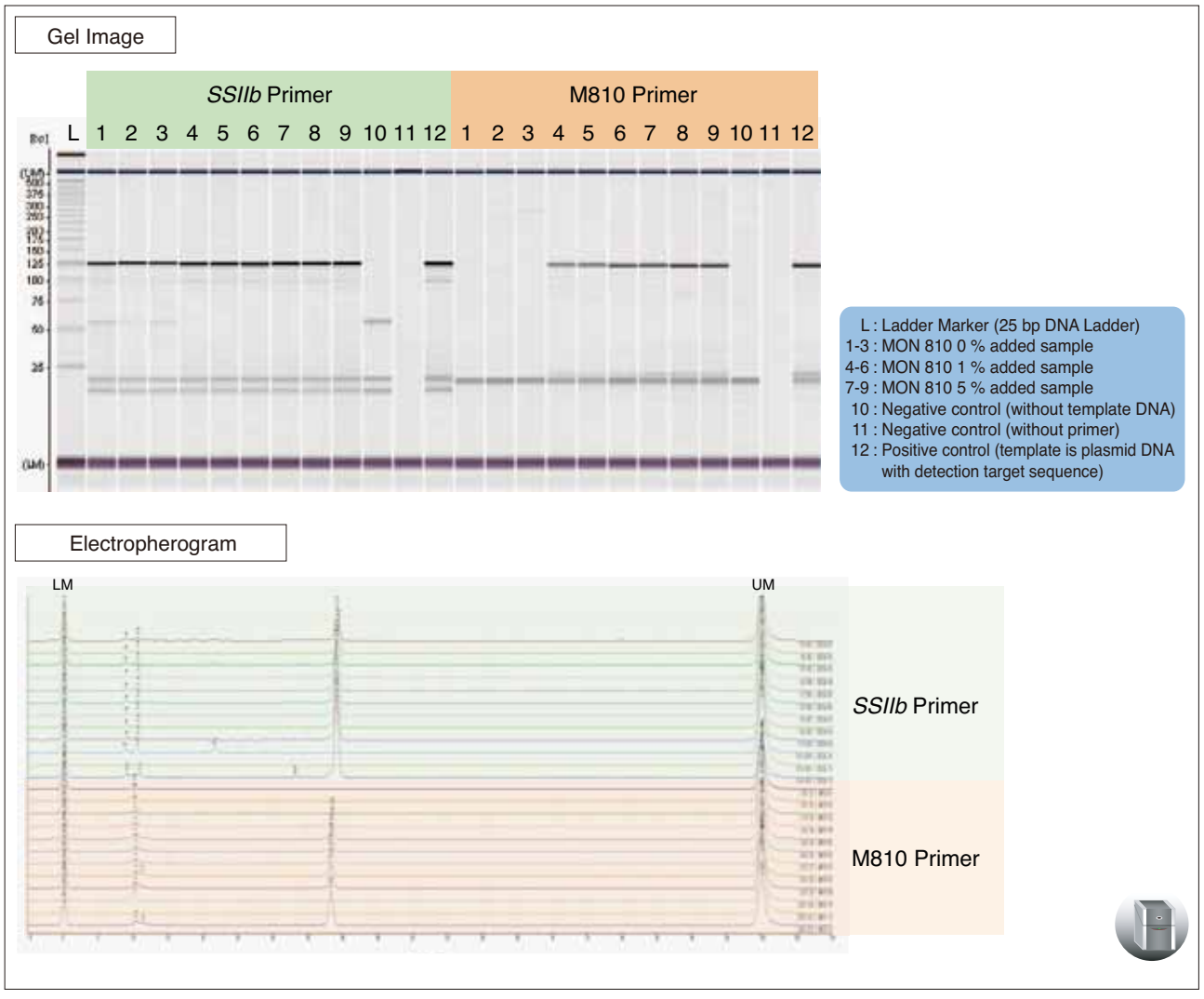


Fig. 9.6.2 Analytical Results for PCR Products from Genetically Modified Corn

9.7 Detection of Mold and Yeast Genes (1) – MCE

■Explanation

Mold and yeast exist throughout the environment and there are many beneficial varieties of each in food sources such as miso (fermented soybean paste), cheese, bread, wine, and sake, etc., and those that are mildly to extremely toxic. Polymerase Chain Reaction (PCR) technology has been used in recent years as one of the molecular biological techniques for detecting fungi such as molds and yeasts. When PCR is performed, a complicated pretreatment operation is typically required to extract the DNA from fungus samples like molds and yeasts. Here we introduce an example of direct mold and yeast analysis using the MCE-202 "MultiNA" DNA/RNA analyzer based on microchip electrophoresis technology, in which the PCR reagent "Ampdirect® Plus" is used to enable the PCR reaction without the need for any PCR pre-processing operations.

■Experimental Procedure

Samples consisted of two types of mold and one type of yeast, as shown in Table 9.7.1. The genus *Eurotium* is a type of mold which grows in dried foods, such as dried goods, bread, filled buns, and jam, etc. The genus *Penicillium*, also referred to as "blue mold", is a genus of mold that occurs in many types of food, such as citrus fruits, grains, and dairy products. There are various types of *Penicillium*, ranging from beneficial varieties that are used in foods such as in the production of cheese, to harmful types such as toxic mold. *Saccharomyces cerevisiae* is budding yeast, and includes such varieties as baker's, wine, and sake yeast. For the PCR reaction reagent, we used Shimadzu's "Ampdirect® Plus Enzyme Set" for gene amplification, and the PCR reaction conditions used are listed in the included instruction manual. The mold and yeast that had been cultured in agar medium were attached to a micropipette tip, then suspended in the PCR reagent solution, and PCR was conducted (Fig. 9.7.1). Primers (ITS primers, for fungi, designed for quick identification of microorganisms by genetic analysis as described in the Japanese Pharmacopoeia(2)) were used for detection of ITS regions(1) when performing PCR. The PCR products were analyzed using the MultiNA. (Fig. 9.7.2)

Table 9.7.1 Molds and Yeast Samples

Molds	: *Eurotium chevalieri*
	Penicillium digitatum
Yeast	: *Saccharomyces cerevisiae*

(1) The Internal Transcribed Spacer (ITS) regions refer to the 2 regions between the 3 ribosomal RNA genes (rDNA), 18 S, 5.8 S and 28 S, (ITS 1 between 18 S and 5.8 S, ITS 2 between 5.8 S and 28 S). It is known that there are differences in the base sequences in these ITS regions among different kinds of bacteria.

(2) The Japanese Pharmacopoeia is book containing standard criteria for pharmaceutical products intended to provide guidelines for establishing standard properties and quality of pharmaceutical products.

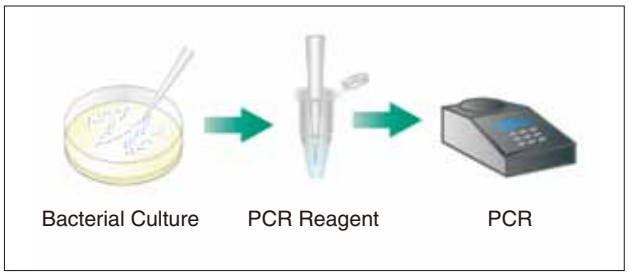


Fig. 9.7.1 Method of Direct PCR

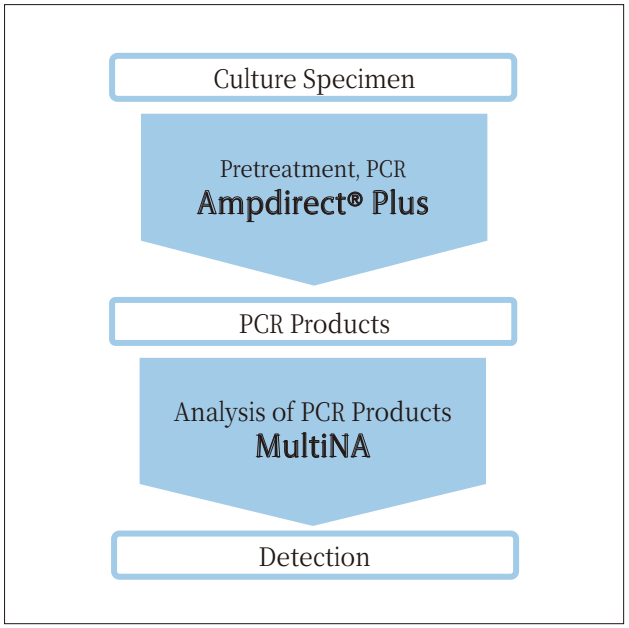


Fig. 9.7.2 Analytical Procedure for Mold and Yeast Genes

9.7 Detection of Mold and Yeast Genes (2) – MCE

■Reagents/Kits

· Ampdirect® Plus Enzyme Set
(Shimadzu Corp.) P/N 241-08890-92
· DNA-500 Kit
(Shimadzu Corp.) P/N 292-27910-91
· SYBR® Gold nucleic acid gel stain
(Invitrogen) S11494
· 25 bp DNA Ladder
(Invitrogen) 10597-011

■Analytical Conditions for PCR Products

Instrument : MCE-202 "MultiNA"
Analysis Mode : DNA-500 Pre-mix mode

■Detection of Mold and Yeast Genes

The analysis results for the mold and yeast gene ITS regions obtained according to the procedures of Fig. 9.7.1 and Fig. 9.7.2 are shown in Fig. 9.7.3. The amplification products specific to the genes of the respective molds and yeast were clearly detected (The size estimations shown in the electropherogram in this figure were obtained through actual experimentation). The measurement results using the MultiNA were obtained as a gel image and electropherogram. In addition, the estimated size values for the amplification products were calculated using a calibration curve generated using a standard sample (Ladder) with known sizes, and the estimated concentration values were calculated based on a high molecular internal standard marker (Upper Marker) of known concentration used as a standard. Thus, evaluation of the target amplification products are detected more reliably and easier than when using agarose gel electrophoresis.

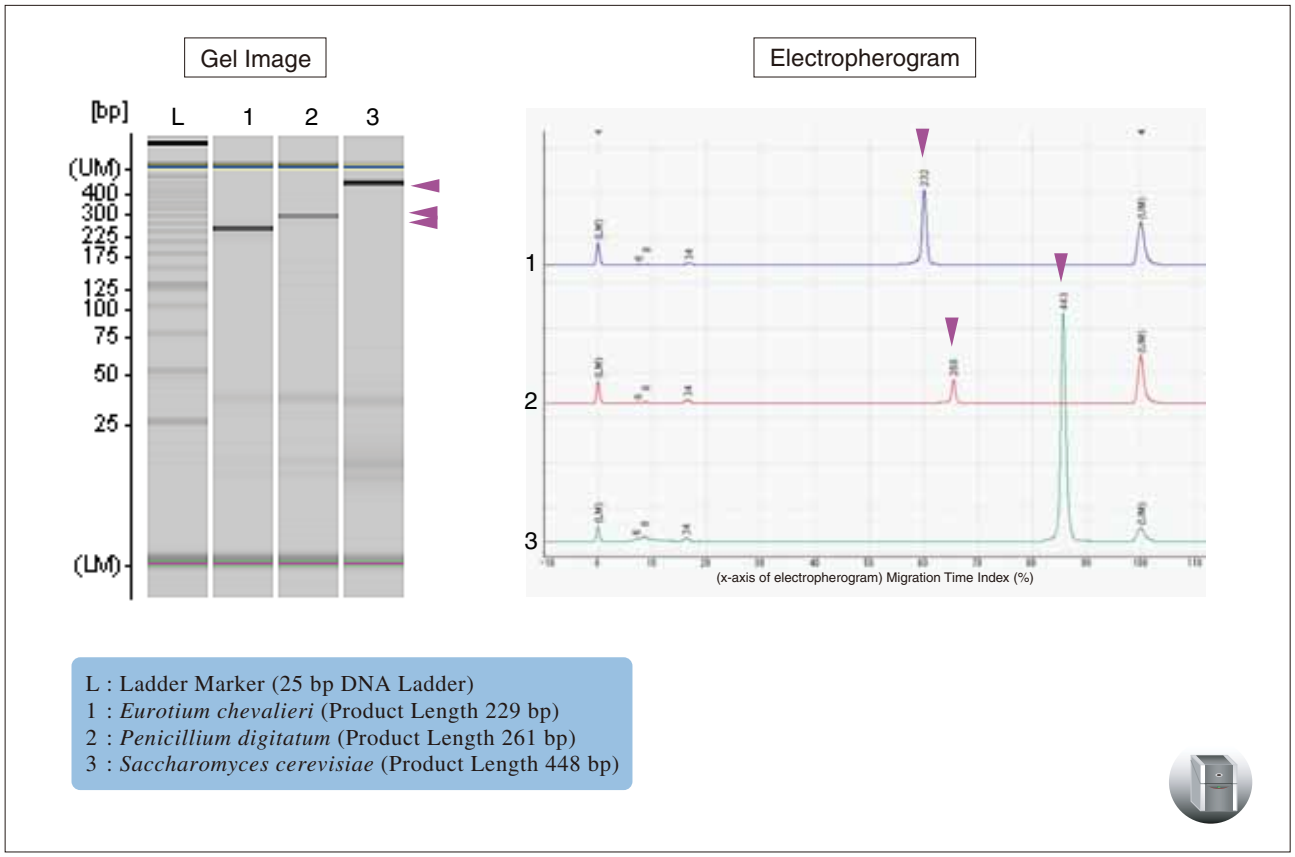


Fig. 9.7.3 Analytical Results for Mold and Yeast Genes

9.8 Detection of Irradiated Foods (1) – GC/MS

■Explanation

Food irradiation is a technology in which foods that are to be stored for long periods of time are exposed to X-rays or gamma rays to kill any microorganisms adhering to the surface of the food. This technology has been used worldwide for such foods as spices and meats, but in Japan, except for the purpose of preventing the germination of potatoes, its use has not been approved. 2-alkylcyclobutanones are formed in the fats contained in food as a result of irradiation. The alkyl groups formed in the 2-alkylcyclobutanones differ depending on the fatty acid composition in the fat; 2-dodecylcyclobutanone (2-DCB) derived from palmitic acid and 2-tetradecylcyclobutanone (2-TCB) derived from stearic acid are known to be principle products generated due to irradiation. Since these are not detected in non-irradiated foods, they are used as radiation detection indicators in the European Standards (EN1785) and in one of the detection methods for irradiated food established by the Japan's Ministry of Health, Labour and Welfare (Alkylcyclobutanone Method; Notice No. 0330 Article 3 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, March 30, 2010). Here we introduce an example of analysis of 2-alkylcyclobutanone in foods from which fat can be extracted (beef, pork, chicken, salmon, and Camembert cheese, etc.) according to the Alkylcyclobutanone Method.

■Materials and Method

Using commercially-available ground pork as samples, one sample was irradiated with 1 kGy of cobalt-60 as the γ-ray source under room temperature, and the other was not irradiated. The sample preparation procedure is shown in Fig. 9.8.2. SIM measurement is required for quantitation in this GC/MS test method, and SCAN measurement is required for qualitative identification. Here, the measurement was conducted in a single analysis using the FASST SCAN/SIM mode, and quantitation of 2-DCB and 2-TCB in the sample was conducted from the SIM measurement results using the internal standard method.

■Analytical Conditions

Instrument	: GCMS-QP2010 Ultra
-GC-	
Column	: Rxi-5MS (30 m × 0.25 mm I.D. df = 0.25 µm)
Column Temp.	: 60 °C (1 min) - 10 °C/min - 300 °C (10 min)
Carrier Gas	: He (46.3 cm/sec, 100 kPa)
Carrier Gas Mode	: Constant Linear Velocity Mode
Injection Temp.	: 250 °C
Injection Method	: Splitless Injection (1 min)
Injection Volume	: 1 µL
-MS-	
I.F. Temp.	: 280 °C
I.S. Temp.	: 230 °C
Ionization Method	: EI
Mode	: SCAN/SIM
Scan Range	: *m/z* 95-115
Scan Interval	: 0.3 sec
Monitor Ion	: *m/z* 98, 112
SIM Interval	: 0.2 sec

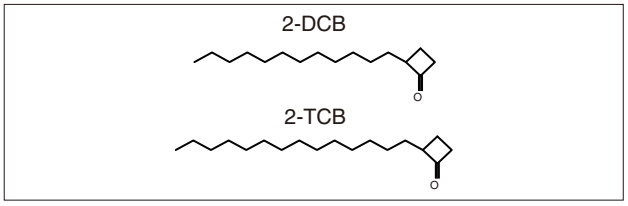


Fig. 9.8.1 Structures of 2-DCB and 2-TCB

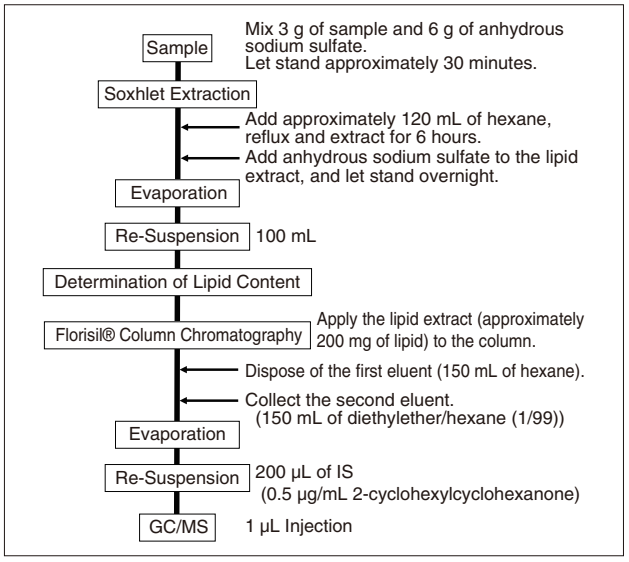


Fig. 9.8.2 Sample Preparation

9.8 Detection of Irradiated Foods (2) – GC/MS

■Sample Analysis

The SIM chromatograms of the irradiated and non-irradiated samples are shown in Fig. 9.8.3 and Fig. 9.8.4, respectively, and the mass spectra of the target analytes in the irradiated samples are shown in Fig. 9.8.5. In the irradiated samples, both 2-DCB and 2-TCB peaks were detected, and their concentrations in fat were 0.077 µg/g and 0.151 µg/g, respectively. The peaks were not detected in the non-irradiated samples. According to the Alkylcyclobutanone Method, if the following 4 conditions are satisfied, the test result is considered to be positive for irradiation of food products.

1) Peaks with S/N ratio greater than 3 are recognized at *m/z* 98 and *m/z* 112 at the same retention times as in the standard solution.
2) The relative ion intensity ratio of *m/z* 112 with respect to *m/z* 98 is within ± 20 % of the relative intensity ratio of a standard solution with a concentration level near that concentration.
3) When SCAN measurement is conducted in the vicinity of the retention time within the range of *m/z* 95 to *m/z* 115, *m/z* 98 and *m/z* 112 are the main ions (total intensity of the 2 ions is at least 50 %).
4) Even if the above items are satisfied, the quantitative values must be greater than the concentration calculated using S/N ratio = 3 of the standard solution.

The irradiated food measurement results obtained here satisfy all of the above criteria, and therefore the results were "positive" for irradiation.

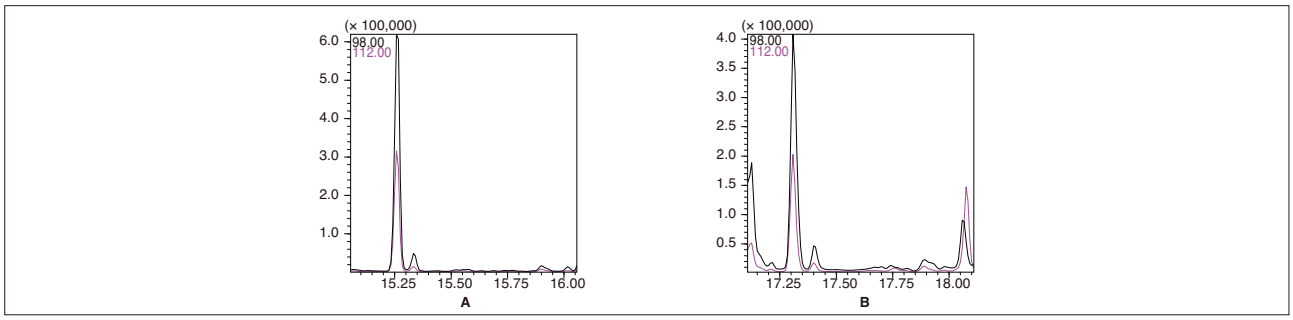


Fig. 9.8.3 SIM Chromatogram of Non-Irradiated Sample

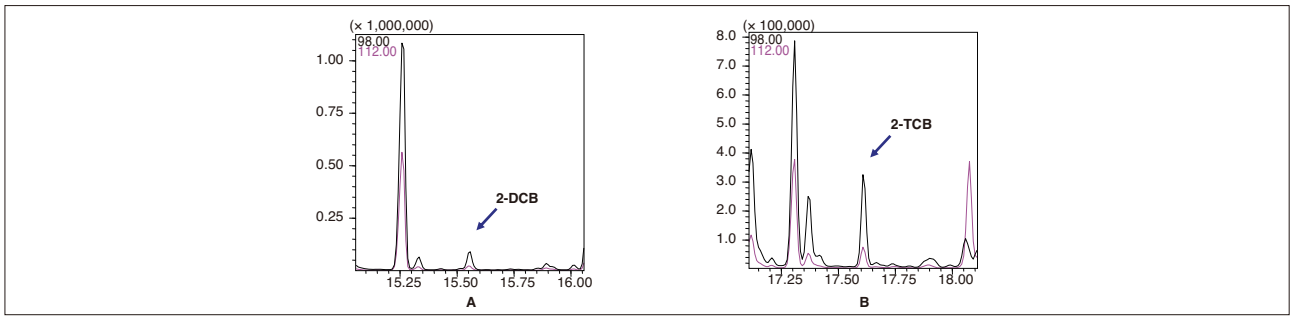


Fig. 9.8.4 SIM Chromatogram of Irradiated Sample

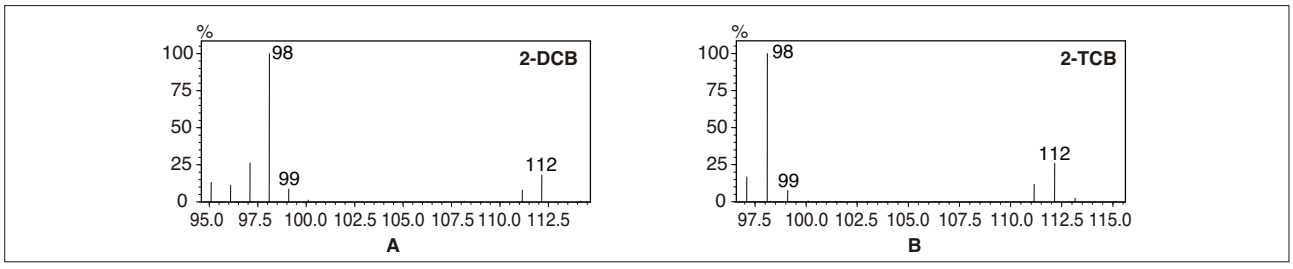


Fig. 9.8.5 Mass Spectra of 2-DCB and 2-TCB (Irradiated Sample)

[Reference]

Notice No. 0330 Article 3 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, March 30, 2010 "Detection Method for Irradiated Foods"

10. Food Containers • Packing Materials

10.1 Leach Test for Phenol in Rubber Nipples (1) – UV

■Explanation

As food moves through the process starting from the raw material stage to the point of ingestion, it comes into contact with a wide variety of items, including implements, containers and packaging materials. These points of physical contact can include equipment used for manufacture and processing, containers and packaging materials used for storage and transport, and cooking and eating utensils used in restaurants and in the home. Because these implements, containers and packaging materials are made of various types of materials, such as rubber, glass, and metals, there is always the possibility that their constituent substances, as well as impurities, can be taken into the body via the ingested food. Therefore, it is necessary to secure the safety of implements, containers, and packaging materials as provided for in the Japan's Food Sanitation Act, "Standards and Criteria for Food and Food Additives, etc., Chapter 3: Apparatus and Containers and Packaging." One example of a rubber implement is the rubber nursing nipple, which is inserted directly in an infant's mouth. Since it is possible that toxic phenol can leach out of the rubber nipple when it is inserted into an infant's mouth, a leach quantity standard has been established in the Food Sanitation Act, and testing is specified to be conducted using an UV-VIS spectrophotometer. Here we introduce the phenol leach test for quantitation of phenol leached from a rubber nipple according to the Food Sanitation Act.

■Standard for Implements, Containers and Packaging for Foods

Table 10.1.1 lists the target analytes and analytical instruments to be used for measurement with respect to the various types of implements and packaging materials. Both general standards and specific standards are specified for rubber and plastic implements, or packaging and containers. Phenol is indicated as an analyte for metal cans, for rubber in the general standard as well as the nursing nipple specific standard, and for phenol plastic, melamine plastic and urea plastic specific standard. In all of these cases, an UV-VIS spectrophotometer is specified for conducting measurement.

■Preparation of Reagents

The various reagents are prepared as described below.

- Phenol standard solution
  Dissolve 1.0 g of phenol in water, and adjust the volume to 100 mL. Take 1 mL of this solution, and add water to bring the volume to 100 mL. Take 1 mL of this solution, and add water to bring the volume to 20 mL. (1 mL of this solution contains 5 μg of phenol.)
- Borate buffer solution
  1st solution: Dissolve 4.0 g of sodium hydroxide in water, and adjust the volume to 100 mL.
  2nd solution: Dissolve 6.2 g of boric acid in water, and adjust the volume to 100 mL.
  Mix equal volumes of the 1st solution and the 2nd solution.
- 4-aminoantipyrine reagent
  Dissolve 1.36 g of 4-aminoantipyrine in water, and adjust the volume to 1000 mL.
- Potassium hexacyanoferrate (Ⅲ)
  Dissolve 8.6 g of potassium hexacyanoferrate (Ⅲ) in water, and adjust the volume to 1000 mL using 1.8 mL of aqueous ammonia (28 to 30 % concentration) and water.

Table 10.1.1 Target Analytes and Analytical Instruments

Implement / Packaging and Container	General /Specific Standard	Target Analyte Substances	Analytical Instrument Note 1)
Glass, earthenware, and enamel	–	Cd, Pb	AA/ICP
Metal cans	–	Cd, Pb	AA/ICP
		Phenol	UV-VIS
		Epichlorohydrin	GC
		Vinyl chloride	GC
Rubber	General standard	Cd, Pb, Zn	AA/ICP
		Phenol	UV-VIS
	Rubber nipples	Cd, Pb, Zn	AA/ICP
		Phenol	UV-VIS
Plastics	General standard	Cd, Pb	AA/ICP
	Phenol plastic Melamine plastic Urea plastic	Phenol	UV-VIS
	Polyvinyl chloride	Dibutyltin compounds	GC/MS
		Cresol phosphate esters	HPLC
		Vinyl chloride	GC
	Polystyrene	Volatile substances (5 types)	GC, GC/MS
	Polyvinylidene chloride	Vinylidene chloride	GC
		Ba	AA/ICP
	Polyethylene terephthalate	Sb, Ge	AA/ICP
	Polymethyl methacrylate	Methyl methacrylate	GC
	Nylon	Caprolactam	GC
	Polycarbonate	Amines	GC
		Bisphenol A, diphenyl carbonate	HPLC
	Polylactate	Total lactate	HPLC

Note 1: AA stands for Atomic Absorption spectrometer,
ICP for inductively coupled plasma emission spectrometer,
UV-VIS for ultraviolet-visible spectrophotometer,
GC for gas chromatograph, GC/MS for gas chromatograph - mass spectrometer, and HPLC for liquid chromatograph.

10.1 Leach Test for Phenol in Rubber Nipples (2) – UV

■Pretreatment and Analytical Conditions

Using a ratio of 1 g of sample to 20 mL of water, immerse the sample in water. Cover the receptacle with a glass plate and set aside for 24 hours, maintaining a temperature of 40 °C. Use this leach solution as the test solution. Add 3 mL of the borate buffer solution to 20 mL of the test solution, and after shaking it well to thoroughly mix the solutions, add 5 mL of the 4-aminoantipyrine solution and 2.5 mL of the potassium hexacyanoferrate (III) solution. Add water to bring the volume to 100 mL, and after shaking well to mix the solutions, set the solution aside for 10 minutes at ambient temperature. Separately, take 20 mL (5 µg/mL) of the phenol standard solution, and perform the same procedure as that for the test solution. Here we used the UV-1800 UV-VIS spectrophotometer, and conducted measurement using the analytical conditions shown in Table 10.1.2.

Table 10.1.2 Analytical Conditions

Photometric Value	Absorbance
Slit Width	1.0 nm
Wavelength Range	300 to 700 nm
Scan Speed	Medium
Sampling Pitch	1 nm

■Results

After preparing test solutions as described above for four types of commercially available rubber nipples, in addition to the standard solution, measurement was conducted using the UV-VIS spectrophotometer. The absorbance spectra obtained for each test solution and the standard solution are shown in Fig. 10.1.1, and the phenol contents indicated by the absorbance values at 510 nm are shown in Table 10.1.3. Extremely low absorbance values were obtained with all of the rubber nipples. With absorbance values lower than that of the specified value for leached phenol, the test solutions here showed almost no leaching of phenol, thereby confirming that the specified standard was satisfied. Here we introduced the phenol leach test for rubber nipples, however, it must be noted that some of the phenol leach conditions are different for types of rubber other than that used in nipples, as well as for metal cans and plastics. In this way, an UV-VIS spectrophotometer can be used for conducting the leach test of phenol from implements, containers, and packaging material used for food.

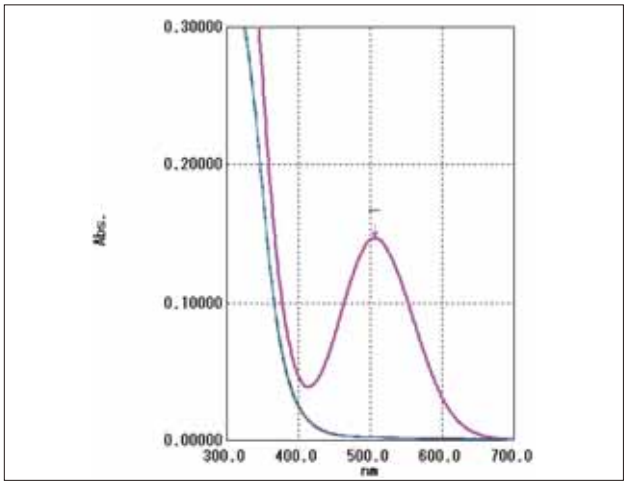


Fig. 10.1.1 Spectra of Standard Solution and Sample Solutions

Table 10.1.3 Results

Sample Name	Absorbance (510 nm)
Company A: isoprene rubber	0.003
Company A: silicone rubber	0.002
Company B: isoprene rubber	0.002
Company B: silicone rubber	0.002
Standard solution	0.146

[Reference]
Author: Yoko Kawamura, "Standards and Criteria for Apparatuses, Containers and Packaging, March 2006 Revision" Chuohoki Publishing

10.2 Analysis of Epichlorohydrin Dissolved from Metal Food Cans – GC

■Explanation

Residual organic solvents in food packaging materials are receiving attention due to the heightened concern for food safety and public health. Specific standards and specifications as well as testing methods are established for each type of material used in food packaging materials in Japan's "Food Sanitation Act-Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc." The inside surface of metal food cans is typically coated with synthetic resin to prevent direct contact of the food with the metal surface of the can. Various types of resins are used for this coating material, including epoxy resin, phenolic resin, and polyvinyl chloride. Separate standards have been established for testing of phenol and formaldehyde, volatile residues, epichlorohydrin, and vinyl chloride; all of these standards rely on dissolution testing followed by gas chromatographic analysis. Here we introduce an example of analysis of epichlorohydrin dissolved from a resin coating on the inside of a can used for canned foods.

■Overview of Epichlorohydrin Analysis Method

This test method provides for GC/FID measurement of epichlorohydrin, one of the raw material monomers of epoxy resin used as an internal-surface coating in metal food cans. (Cans that are used for dried foods or solid foods that make little direct contact with the can's inside surface, and foods other than oily and fatty foods are exempt from this testing. However, cans that are manufactured with the intention of storing sterilized or pasteurized foods for a long period of time, regardless of the type of food, are subject to this testing.) In the dissolution test, *n*-pentane is used as the dissolving solution, and the peak area value of epichlorohydrin in the test solution is checked to ensure that it is not greater than the peak area of epichlorohydrin standard solution (0.5 µg/mL).

■Analytical Method

Sample preparation was conducted according to that specified in the "Food Sanitation Act-Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc." Using a commercially-available food can as the sample, the *n*-pentane dissolved solution was heated in the can at 25 °C for 1 hour to allow dissolving, and the obtained dissolved solution was used as the test solution. Since epichlorohydrin and *n*-pentane evaporate easily, the can was covered with aluminum foil and polyvinyl chloride wrapping film, and the film was fixed in place with a rubber band to seal in the solution. A RESTEK Rtx-WAX (30 mL, 0.53 mm ID, 1 µm film thickness) was used. The carrier gas flowrate was set so that epichlorohydrin would elute in about 7 minutes. The sample preparation flow chart is shown in Fig. 10.2.1.

■Analytical Conditions

Instrument	: GC-2010 Plus AF
Column	: Rtx-WAX (30 m × 0.53 mm I.D. df = 1 µm)
Column Temp.	: 50 °C (5 min) - 10 °C/min - 100 °C
Carrier Gas	: He 74 cm/sec (Constant Linear Velocity Mode)
Injection Temp.	: 220 °C
Injection Method	: Split Injection
Split Ratio	: 1:10
Injection Volume	: 5.0 µL
Detector	: FID
Detector Temp.	: 220 °C

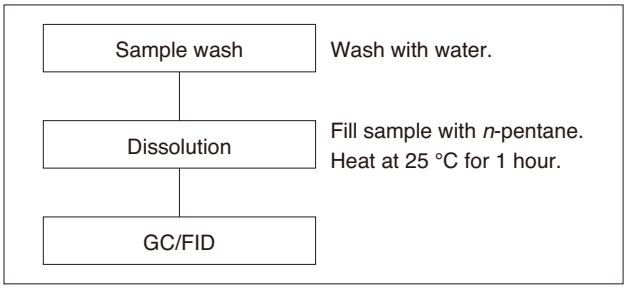


Fig. 10.2.1 Preparation of Metal Food Can

■Analysis of Standard Solution and Test Solution

The chromatograms of the epichlorohydrin standard solution and the test solution obtained using a commercially-available metal food can are shown in Fig. 10.2.2. The epichlorohydrin peak in the test solution chromatogram has a smaller peak area than that of the standard solution, confirming that the concentration of the sample is smaller than the reference value.

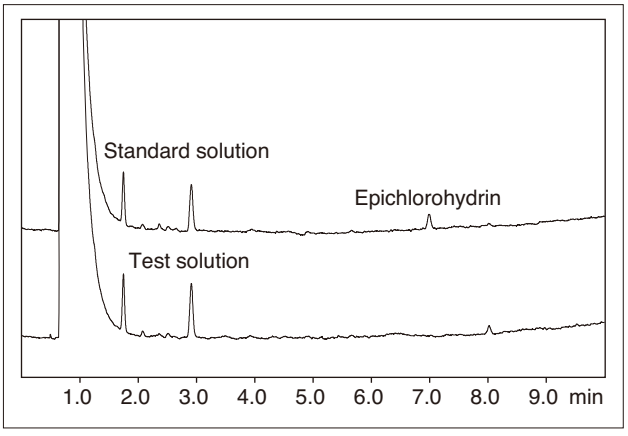


Fig. 10.2.2 Chromatograms of Epichlorohydrin Standard Solution and Test Solution

[Reference]

March 31, 2006 Ministry of Health, Labour and Welfare Notification No. 201

Food Sanitation Act - Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc.

10.3 Analysis of Triethylamine and Tributylamine in Polycarbonate Plastics (1) – GC

■Explanation

Specific standards and criteria as well as testing methods were established for each type of material used in food packaging materials in the Japan's "Food Sanitation Act - Section 3: Implements and Container and Packaging in the Standards and Criteria for Food and Food Additives, etc." Polycarbonate is a transparent plastic material which possesses excellent mechanical strength, especially impact resistance and heat resistance. It is used in a wide variety of utensils in the food industry: nursing bottles, tableware, chopsticks, mugs, and drip coffee makers, to name a few. Individual standards have been established for testing of specific polycarbonate constituents, including bisphenol A, diphenyl carbonate, amines (triethylamine and tributylamine). In addition, testing procedures have been specified for leach testing for bisphenol A and residues after evaporation. Here we introduce an example of analysis of amines (triethylamine and tributylamine), two of the principle ingredients in polycarbonate plastic.

■Overview of Triethylamine and Tributylamine Analysis in Polycarbonate Plastics

This test method consists of dissolving the polycarbonate and extracting any existing triethylamine and tributylamine. Measurement of these constituents is then conducted by Gas Chromatography with a nitrogen-specific Flame Thermionic Detector (GC-FTD). The test method consists of verifying that the total content of triethylamine and tributylamine in the sample does not exceed 1 μg/g.

■Sample Preparation

Sample pretreatment was conducted according to that specified in the "Food Sanitation Act - Section 3: Implements and Container and Packaging in the Standards and Criteria for Food and Food Additives, etc." A commercially available garlic press made of polycarbonate was used for the sample. About 1 g of sample was accurately weighed and transferred to a 200 mL conical flask, and 20 mL of dichloromethane was then added to the flask. After sample dissolution, 100 mL acetone was added while mixing well, and centrifugation at 3000 rpm was conducted for 10 minutes to separate the phases. After concentrating the supernatant to 1 mL using a vacuum concentrator, dichloromethane was added to bring the total volume to 2 mL. This was used as the test solution. The sample preparation procedure is shown in the flow chart of Fig. 10.3.1.

■Analytical Conditions

Instrument	: GC-2010 Plus AF+FTD-2010 Plus
Column	: Rtx-1 (30 m × 0.32 mm I.D. df = 5 μm)
Column Temp.	: 150 °C (5 min) - 20 °C/min - 250 °C (5 min)
Carrier Gas	: He 32.5 cm/sec (Constant Linear Velocity Mode)
Injection Temp.	: 200 °C
Injection Method	: Split Injection
Split Ratio	: 1:15
Injection Volume	: 1.0 μL
Detector	: FTD
Detector Temp.	: 250 °C

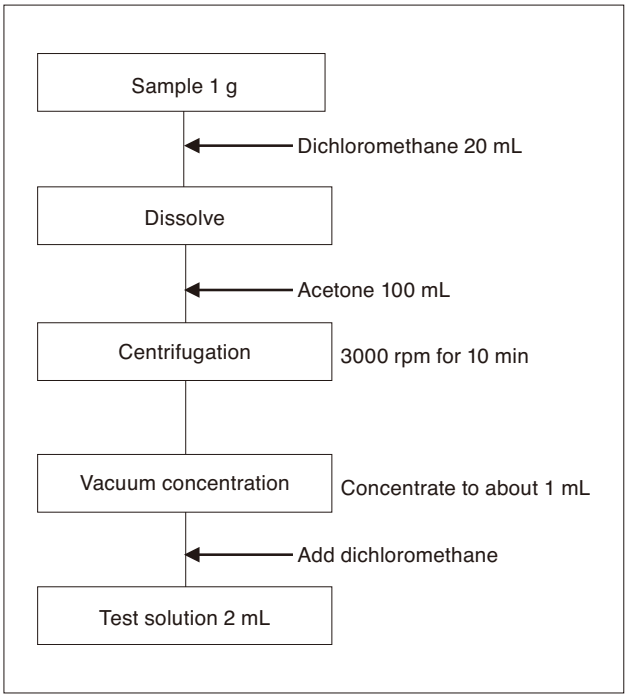


Fig. 10.3.1 Preparation of Polycarbonate Plastic Sample (Analysis of Amines)

10.3 Analysis of Triethylamine and Tributylamine in Polycarbonate Plastics (2) – GC

■Calibration Curves

A stock solution of 0.1 mg/mL of triethylamine and tributylamine in dichloromethane was prepared. Serial dilutions of the stock solution were made to produce standard solutions (0.2, 0.4, 0.6, 0.8, 1.0 µg/mL). Measurement of each solution was conducted by GC using a 1 µL injection volume, and the calibration curves were generated from the peak areas of triethylamine and tributylamine.

■Analysis of Standard Solution and Sample Solution

The chromatograms of the triethylamine and tributylamine standard solution (0.2 µg/mL) and the test solution of the processed polycarbonate garlic press sample are shown in Fig. 10.3.3. Although triethylamine was detected in the chromatogram of the test solution, the content was less than one-third that of the specified standard value.

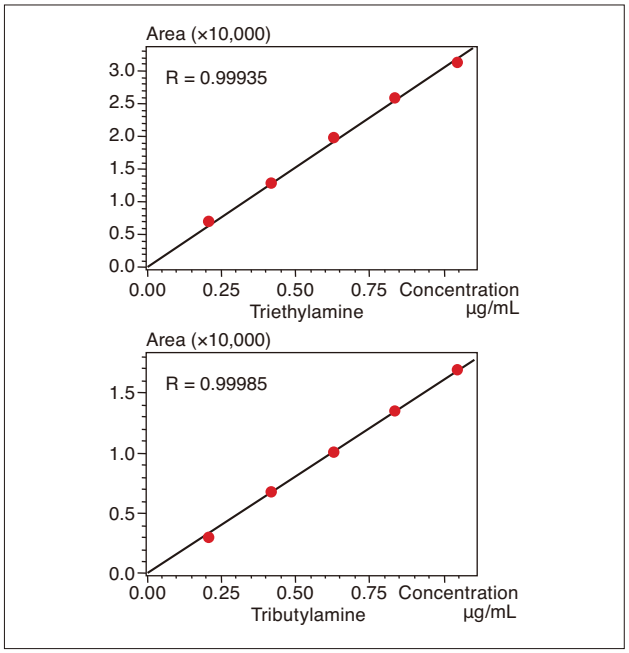


Fig. 10.3.2 Calibration Curves of Triethylamine and Tributylamine (0.2 to 1.0 µg/mL)

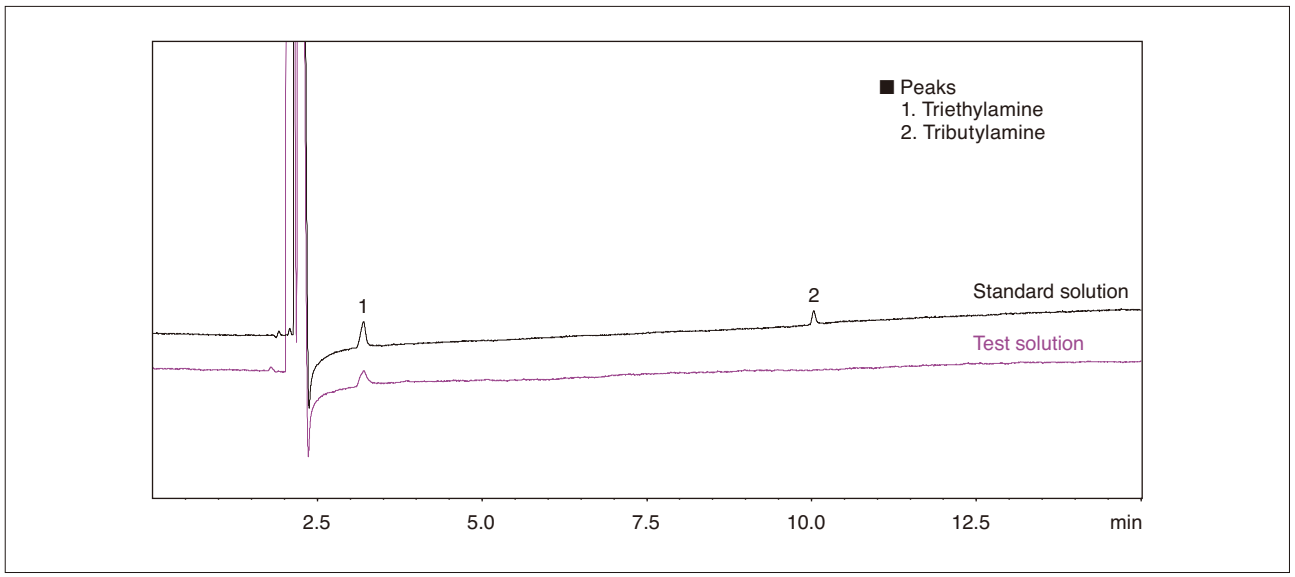


Fig. 10.3.3 Chromatograms of Standard Solution (Triethylamine, Tributylamine 0.2 µg/mL each) and Test Solution

Note:
Consumption of the alkali source of the FTD when using dichloromethane as the sample solvent is slightly faster compared to that when other (hydrocarbon) solvents are used. Details are described in the FTD instruction manual.

[Reference]
March 31, 2006 Ministry of Health, Labour and Welfare Notification No. 201
Food Sanitation Act - Section 3: Implements and Container and Packaging in the Standards and Criteria for Food and Food Additives, etc.

10.4 Analysis of Vinylidene Chloride in Polyvinylidene Chloride Plastics – GC

■Explanation

Specific standards and specifications as well as testing methods are established for each type of material used in food packaging materials in Japan's "Food Sanitation Act-Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc." Polyvinylidene chloride is a polymer that is transparent, displays excellent water resistance, chemical resistance, and gas-barrier properties, and is resistant to temperatures in the range of 140 to 170 °C. In addition to household wrapping film, it is used as a film for wrapping foods that have been heated to high temperatures. Vinylidene chloride is the monomer of polyvinylidene chloride, and long-term oral exposure is said to adversely affect the liver and kidney. Due to the possibility that vinylidene chloride may persist in products, polyvinylidene chloride material testing has been established as a separate standard in the Food Sanitation Act. Here we introduce an example of analysis of vinylidene chloride in plastic

■Analytical Method

The vinylidene chloride test method is for measuring vinylidene chloride monomer present in polyvinylidene chloride by GC/FID using the headspace method. Sample pretreatment is conducted according to that specified in the "Food Sanitation Act-Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc." Commercially-available polyvinylidene chloride household wrapping film was used as the sample, and the vinylidene chloride in the sample was analyzed. A porous polymer PLOT (porous layer open tubular) CP-PoraBOND Q column was used. The carrier gas flowrate was set so that the vinylidene chloride would elute at about 9 minutes. Following is an overview of the analytical procedure.

(1) Test Solution Preparation
Cut and weigh out a 0.5 g fragment of sample, and transfer it to a headspace vial. Next, add 2.5 mL *N*,*N*-dimethylacetamide, seal the vial, and use this as the test solution.

(2) Standard Solution Preparation
Transfer 50 µL vinylidene chloride standard solution (60 µg/mL) to a vial containing 2.5 mL *N*,*N*-dimethylacetamide, and seal the vial. Use this as the standard solution.

(3) Measurement
Heat the sealed vials of test solution and standard solution for 1 hour at 90 °C, and introduce 0.5 mL of the respective gas phases into the gas chromatograph. For the gas chromatograph column, use a 3 µm styrene-divinylbenzene porous polymer-coated column, and conduct analysis by GC/FID.

(4) Determination
Compare the detection times of test solution peak and the vinylidene chloride standard solution peak. If the retention times correspond, compare their respective peak areas. Verify that the test solution vinylidene chloride peak area is not greater than that of the vinylidene chloride standard solution peak area (6 µg/g or less in the material).

■Analysis of Standard Solution and Sample Solution

The chromatograms of the vinylidene chloride standard solution and the test solution prepared from commercially-available household wrapping film, of which the principle ingredient is polyvinylidene chloride, are shown in Fig. 10.4.1. The vinylidene chloride peak in the test solution chromatogram has a smaller peak area than that of the standard solution, confirming that it is smaller than the reference value.

■Analytical Conditions

Instruments	: TurboMatrix HS-40 + GC-2010PlusAF
Column	: CP-PoraBOND Q FUSED SILICA (25 m × 0.25 mm I.D. df = 3 µm)
Column Temp.	: 80 ˚C (1 min) - 10 ˚C/min - 250 ˚C (10 min)
Injection Temp.	: 200 ˚C
Carrier Gas	: He 30 cm/sec
Detector	: FID
Detector Temp.	: 250 ˚C
Injection Volume	: 0.5 mL
Sample Thermostatting	: 90 ˚C, 60 min

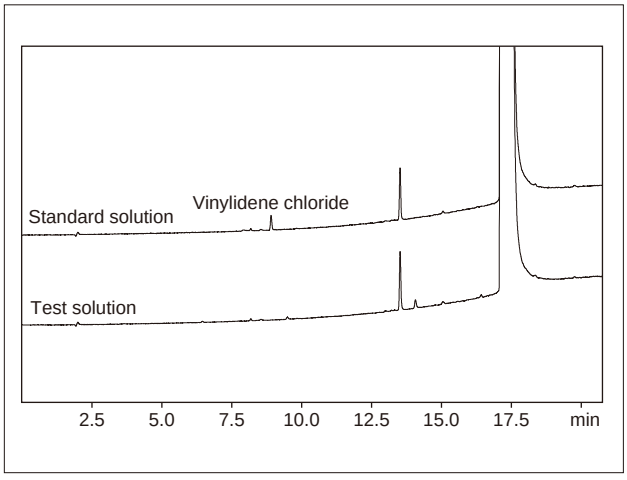


Fig. 10.4.1 Chromatograms of Vinylidene Chloride Standard Solution and Test Solution

[Reference]

March 31, 2006 Ministry of Health, Labour and Welfare Notification No. 201
Food Sanitation Act - Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc.

10.5 Analysis of Vinyl Chloride in Polyvinyl Chloride Plastics – GC

■Explanation

Specific standards and specifications as well as testing methods are established for each type of material used in food packaging materials in Japan's "Food Sanitation Act-Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc." Polyvinyl chloride, a transparent plastic, can be easily mixed with plasticizers to obtain a desired degree of flexibility depending on the compounding ratio. For that reason, it is used in a variety of applications, including food containers, wrapping films, and gloves. Vinyl chloride is the monomer of polyvinyl chloride, and is generated when polyvinyl chloride is subjected to thermal decomposition. Due to its widely-reported carcinogenicity, the concentration of vinyl chloride in materials is restricted in conjunction with material testing. Here we introduce an example of analysis of vinyl chloride in plastic with polyvinyl chloride as its principal ingredient.

■Analytical Method

The vinyl chloride test method is for measuring vinyl chloride monomer present in polyvinyl chloride by GC/FID using the headspace method. Sample pretreatment is conducted according to that specified in the "Food Sanitation Act - Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc." A commercially-available polyvinyl chloride glove was used as the sample, and the vinyl chloride in the sample was analyzed. A porous polymer PLOT (porous layer open tubular) CP-PoraBOND Q column was used. The carrier gas flowrate was set so that the vinyl chloride would elute at about 5 minutes. Following is an overview of the analytical procedure.

(1) Test Solution Preparation
Cut and weigh out a 0.5 g fragment of sample, and transfer it to a headspace vial. Next, add 2.5 mL *N*,*N*-dimethylacetamide, seal the vial, and use this as the test solution. (If it is difficult to dissolve the sample, shake the sealed vial occasionally at ambient temperature, and allow it to stand overnight.)

(2) Standard Solution Preparation
Transfer 50 µL vinyl chloride standard solution (10 µg/mL) (cooled using methanol and dry ice) to a vial containing 2.5 mL *N*,*N*-dimethylacetamide, and immediately seal the vial. Use this as the standard solution.

(3) Measurement
Heat the sealed vials of test solution and standard solution for 1 hour at 90 °C, and introduce 0.5 mL of the respective gas phases into the gas chromatograph. For the gas chromatograph column, use a 3 µm styrene-divinylbenzene porous polymer-coated column, and conduct analysis by GC/FID.

(4) Determination
Compare the detection times of test solution peak and the vinyl chloride standard solution peak. If the retention times correspond, compare their respective peak areas. Verify that the test solution vinyl chloride peak area is not greater than that of the vinyl chloride standard solution peak area (1 µg/g or less in the material).

■Analysis of Standard Solution and Sample Solution

The chromatograms of the vinyl chloride standard solution and the test solution prepared from a commercially-available synthetic polymer glove, of which the principle ingredient is polyvinyl chloride, are shown in Fig. 10.5.1. The vinyl chloride peak in the test solution chromatogram has a smaller peak area than that in the standard solution, confirming that it is smaller than the reference value.

■Analytical Conditions

Instruments	: TurboMatrix HS-40 + GC-2010PlusAF
Column	: CP-PoraBOND Q FUSED SILICA (25 m × 0.25 mm I.D. df = 3 µm)
Column Temp.	: 80 ˚C (1 min) - 10 ˚C/min - 250 ˚C (10 min)
Injection Temp.	: 200 ˚C
Carrier Gas	: He 28.7 cm/sec
Detector	: FID
Detector Temp.	: 250 ˚C
Injection Volume	: 0.5 mL
Sample Thermostatting	: 90 ˚C, 60 min

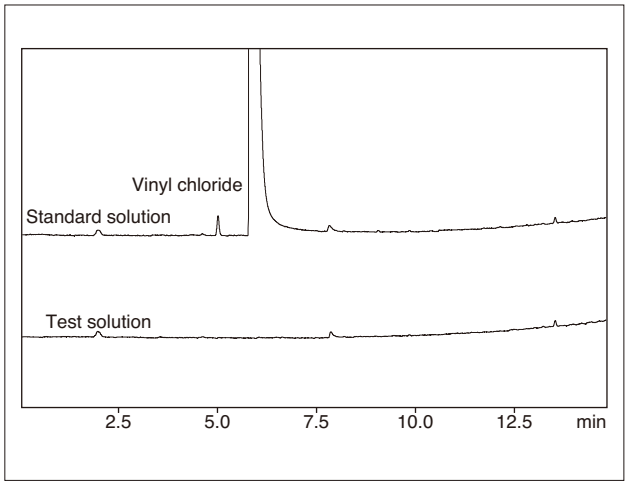


Fig. 10.5.1 Chromatograms of Vinyl Chloride Standard Solution and Test Solution

[Reference]
March 31, 2006 Ministry of Health, Labour and Welfare Notification No. 201
Food Sanitation Act - Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc.

10.6 Analysis of Methyl Methacrylate in Polymethyl Methacrylate Plastics – GC

■Explanation

Due to the high level of concern for food safety, attention is being focused on residual organic solvents in food packaging. Utensils and food packaging materials are subject to standards controlled through material testing as specified in Japan's Food Sanitation Act-Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc. Polymethyl methacrylate is primarily used in table articles such as chopsticks, cups, soy sauce dispensers, etc. A separate food packaging standard is provided for the monomer methyl methacrylate. Here we introduce an example of analysis of methyl methacrylate in plastics in which polymethyl methacrylate is the main ingredient.

■Overview of Methyl Methacrylate Analysis in Polymethyl Methacrylate Plastics

This standard covers mainly polymethyl methacrylate synthetic resins, in which the base polymer consists of at least 50 % methyl methacrylate. Polymethyl methacrylate is primarily used in table articles due to its high transparency, good weather resistance and excellent machinability. A separate leachate testing standard is provided for methyl methacrylate, the monomer of polymethyl methacrylate. The methyl methacrylate test method specifies the use of GC/FID to measure the amount of methyl methacrylate that leaches from the sample immersed in a 20 % aqueous ethanol solution. In the leaching test, the peak area value of methyl methacrylate in the test solution is not to exceed the peak area value obtained from analysis of a 15 µg/mL methyl methacrylate standard solution.

■Analytical Method

Sample preparation was conducted according to that specified in Japan's Food Sanitation Act-Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc. Commercially available chopsticks made of polymethyl methacrylate synthetic resin were used for the testing. For the leaching test, a piece of a chopstick with a 1 cm^2 surface area was immersed in 2 mL of 20 % aqueous ethanol solution and maintained at 60 °C for 30 minutes for leaching, and the resulting solution was used as the test solution. A Restek Rtx-1 capillary column (0.32 mm I.D., 30 m length, 5 µm film thickness) was used, and analysis was conducted by GC/FID. The preparation procedure is shown in the flow chart of Fig. 10.6.1.

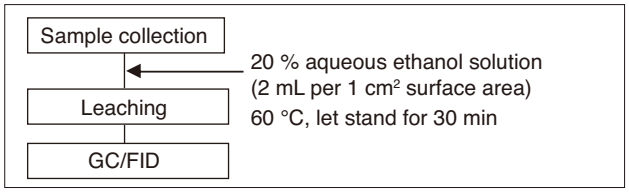


Fig. 10.6.1 Preparation of Polymethyl Methacrylate Plastic Sample

■Analytical Conditions

Instrument	: GC-2010 Plus AF
Column	: Rtx-1 (30 m × 0.32 mm I.D. df = 5 µm)
Column Temp.	: 120 °C (1 min) - 5 °C/min - 170 °C
Carrier Gas	: He 29.5 cm/sec (Constant Linear Velocity Mode)
Injection Temp.	: 200 °C
Injection Method	: Split Injection
Split Ratio	: 1:10
Injection Volume	: 1.0 µL
Detector	: FID
Detector Temp.	: 200 °C

■Analysis of Standard Solution and Test Solution

Fig. 10.6.2 shows the chromatograms obtained from analysis of a methyl methacrylate standard solution (15 µg/mL) and the test solution prepared using a commercially available chopstick made of polymethyl methacrylate synthetic resin. The peak area value of methyl methacrylate in the chromatogram test solution is clearly smaller than that in the standard solution, which confirms that the test value satisfies the standard criterion.

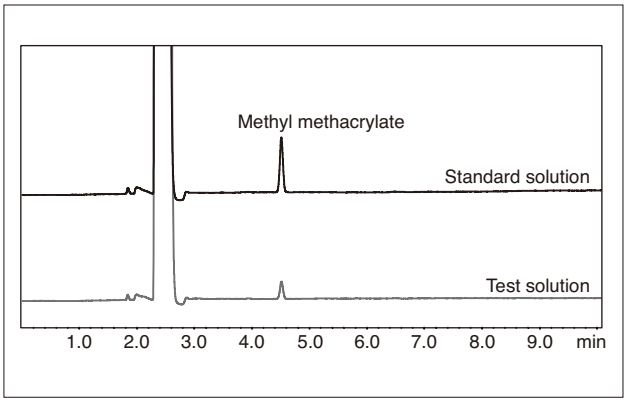


Fig. 10.6.2 Chromatograms of Standard Solution (15 µg/mL) and Test Solution

[Reference]

Notification No. 201 of the Ministry of Health, Labour and Welfare, March 31, 2006
Food Sanitation Act-Section 3: Implements, Containers, and Packaging in the Standards and Criteria for Food and Food Additives, etc.

10.7 Analysis of Heavy Elements in Tableware (1) – ICP-AES

■Explanation

Kitchen utensils and containers regularly come in direct contact with food articles, and therefore can contaminate food with toxic heavy metals, etc. through migration, thus posing a health hazard. In particular, ceramic and glass tableware may be decorated and coated with pigments and glaze which contain toxic lead and cadmium, and physical injury due to migration of these toxic elements becomes a problem. Based on this, the Ministry of Heath, Labour and Welfare of Japan revised the Food Sanitation Act "Standards for Foods and Additives" on July 31, 2008 with respect to Notification No. 370 of the Ministry of Health, Labour and Welfare (1959). The main points of revision concerning tableware are (1) standardization according to the material, capacity, and shape of containers, and (2) strengthening of the standard for migration of cadmium and lead in accordance with ISO. Table 10.7.1 shows the revised specification standards according to material. The inspection method is a migration test, in which the heavy metal is placed in contact with 4 % acetic acid, assuming vinegar as the easiest dissolution food substance, for 24 hours at ambient temperature to allow migration into the acid solution. Quantitative analysis of the solution is conducted by atomic absorption spectrophotometry or ICP emission spectrometry. ICP-AES (ICP emission spectrometry) is a high-sensitivity technique that allows simultaneous analysis of multiple elements. Here we introduce the analysis of commercial tableware using the Shimadzu ICPE-9000 multi-type emission spectrometer.

Table 10.7.1 Revised Specifications According to Material Category

Material	Classification		Cd	Pb
Glass	Sample which cannot be filled with liquid, or sample whose depth is less than 2.5 cm		0.7 μg/cm^2	8 μg/cm^2
	Sample whose depth is 2.5 cm or greater when filled with liquid			
	Other than heat-cookware	Capacity less than 600 mL	0.5 μg/mL	1.5 μg/mL
		Capacity 600 mL or more, and less than 3 L	0.25 μg/mL	0.75 μg/mL
		Capacity 3 L or more	0.25 μg/mL	0.5 μg/mL
	Heat-cookware		0.05 μg/mL	0.5 μg/mL
Ceramic	Sample which cannot be filled with liquid, or sample whose depth is less than 2.5 cm		0.7 μg/cm^2	8 μg/cm^2
	Sample whose depth is 2.5 cm or greater when filled with liquid			
	Other than heat-cookware	Capacity less than 1.1 L	0.5 μg/mL	2 μg/mL
		Capacity 1.1 L or more, and less than 3 L	0.25 μg/mL	1 μg/mL
		Capacity 3 L or more	0.25 μg/mL	0.5 μg/mL
	Heat-cookware		0.05 μg/mL	0.5 μg/mL
Porcelain Enamel	Sample which cannot be filled with liquid, or sample whose depth is less than 2.5 cm			
	Other than heat-cookware		0.7 μg/cm^2	8 μg/cm^2
	Heat-cookware		0.5 μg/cm^2	1 μg/cm^2
	Sample whose depth is 2.5 cm or greater when filled with liquid			
		Capacity 3 L or more	0.5 μg/cm^2	1 μg/cm^2
	Other than heat-cookware	Capacity less than 3 L	0.07 μg/mL	0.8 μg/mL
	Heat-cookware		0.07 μg/mL	0.4 μg/mL

10.7 Analysis of Heavy Elements in Tableware (2) – ICP-AES

■ Samples

Commercial tableware (glass, ceramic)

■Sample Preparation

(According to Standards for Foods and Additives)
After washing the sample well with water, fill it with 4 % acetic acid solution (v/v), and leave it at ambient temperature in a dark place for 24 hours. Use this solution as the test solution.

■Results

Fig. 10.7.1 shows the ICPE-9000 spectral profiles, and Table 10.7.2 shows the quantitation results and detection limit of this analysis. The detection limit in this analysis is less than 1/100 of the standard value, demonstrating high sensitivity.

■Analytical Conditions

Instrument	: ICPE-9000
RF Output	: 1.2 (kW)
Plasma Gas Flowrate	: 10 (L/min)
Auxiliary Gas Flowrate	: 0.6 (L/min)
Carrier Gas Flowrate	: 0.60 (L/min)
Sample Introduction	: Coaxial nebulizer
Spray Chamber	: Cyclone chamber
Plasma Torch	: Mini torch
Observation Method	: Axial

Table 10.7.2 Quantitative Results for Tableware (Unit: µg/mL)

Element Name		Cadmium (Cd)	Lead (Pb)
Detection Limit		0.0001	0.002
Sample Name	Sample Type		
Sample 1	Glass cup with pattern design	0.0002	< 0.002
Sample 2	Earthenware cup	< 0.0001	0.020
Sample 3	Earthenware saucer	< 0.0001	< 0.002
Sample 4	Porcelain teacup with pattern design	0.0010	0.056
Sample 5	Small porcelain pot with pattern design	0.0027	0.221
Sample 6	Porcelain coffee cup	< 0.0001	0.061

<: Detection limit (3σ) obtained from the standard deviation of N=10 repeat measurements using a calibration curve blank (4 % acetic acid solution (v/v))

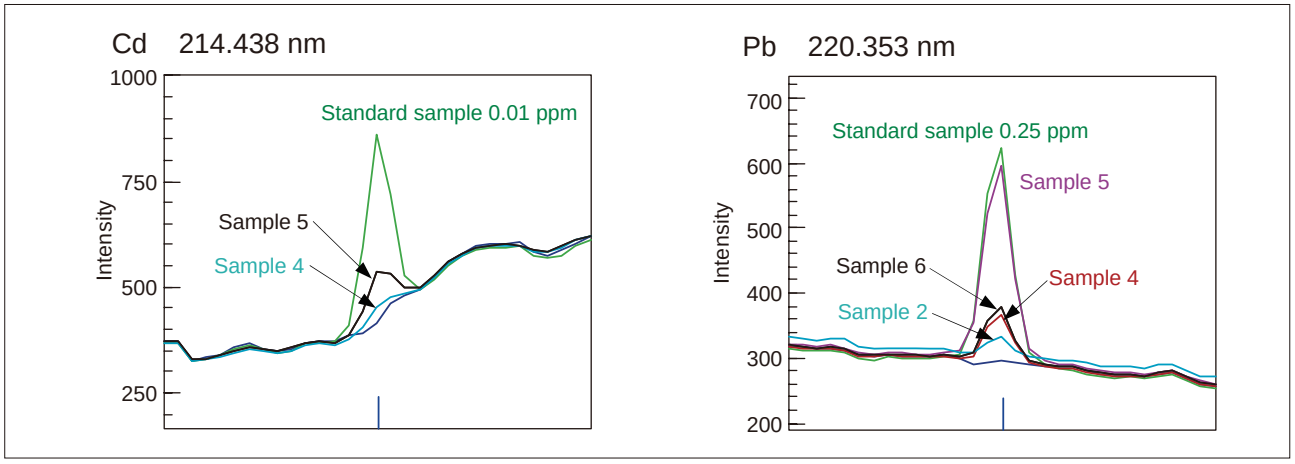


Fig. 10.7.1 Spectral Profiles of Tableware

10.8 Analysis of Heavy Elements in a Cup – EDX

■Explanation

Lead, a harmful element, has recently been detected in toys, household articles, and everyday products, and the safety of these articles is becoming a problem. Since solid, powder, and liquid samples can be analyzed nondestructively, both quickly and easily by X-ray fluorescence analysis, this method is used as a screening technique for safety inspections of such items as toys and household articles. Here we introduce the results of analysis of a dishware article containing harmful elements. A polyethylene (PE) resin (plastic) spiked with 8 harmful elements was used as a standard sample.

■Analysis of Cup

The photographs of the cup (Fig. 10.8.1) indicate the locations where measurement was conducted on the yellow coating site. The qualitative results of Fig. 10.8.2 indicate the presence of Pb in the yellow portion of the cup. In addition, the quantitative analysis results for the (1) yellow, (2) red, (3) green, and (4) white portions of coating are shown in Table 10.8.1. The quantitative analysis results indicate that aside from Pb, the elements Cr and Cd are also present.

■Analytical Conditions

Instrument	: EDX-720
X-ray Tube	: Rh target
Filter	: Filter #4 (for Cd, Sb, Ba), Filter #3 (for As, Hg, Pb, Se), Filter #1 (for Cr)
Voltage	: 50 kV except for Cr, Cr: 30 kV
Current	: Auto
Atmosphere	: Air
Measurement Diameter	: 10 mm ϕ
Measurement Time	: 100 sec
Dead Time	: 40 %

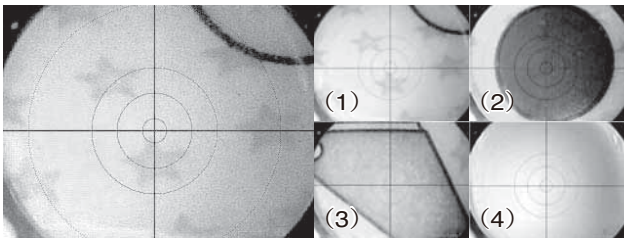


Fig. 10.8.1 Photographs of the cup

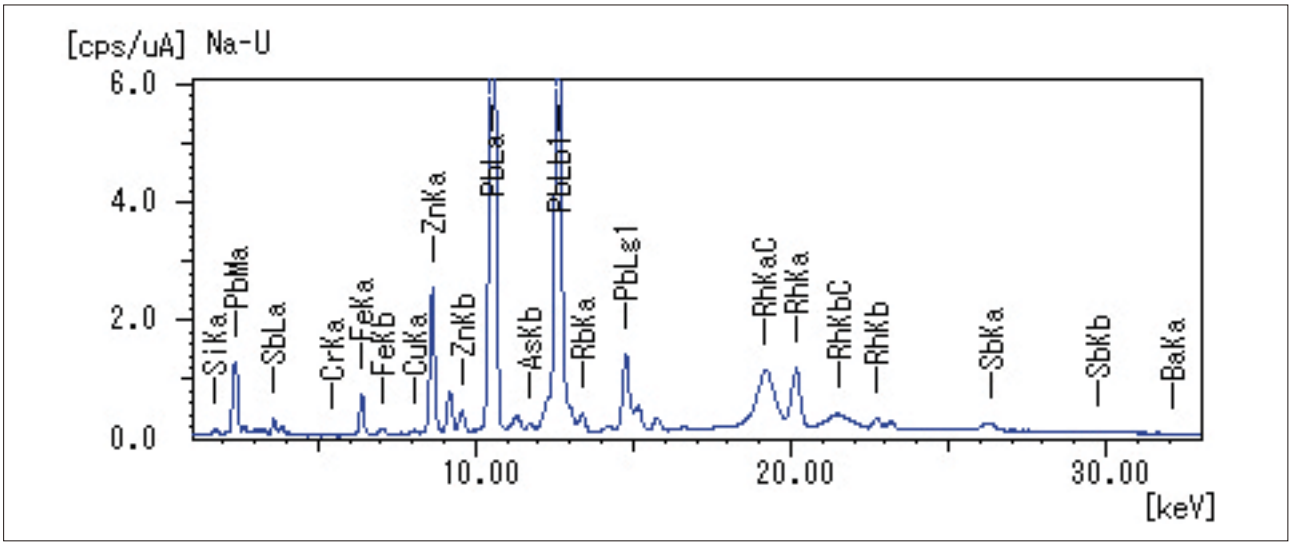


Fig. 10.8.2 Qualitative Analysis Results for Yellow Coating of Cup

Table 10.8.1 Quantitative Analysis Results for Cup

Element	Sb	As	Ba	Cd	Cr	Pb	Hg	Se
Spectrum	Sb Kα	As Kα	Ba Kα	Cd Kα	Cr Kα	Pb Lβ1	Hg Lα	Se Kα
(1) Yellow color	743.9	166.1	72.5	11.7	113.9	25554.2	N. D.	72.0
(2) Red	218.2	22.3	112.0	1207.3	15.6	9094.6	N. D.	1327.0
(3) Green	106.1	101.2	91.2	N. D.	4741.8	11643.3	N. D.	38.2
(4) White	N. D.	N. D.	111.2	N. D.	N. D.	80.2	N. D.	N. D.

(Unit: ppm)

10.9 X-Ray CT Observation of Seals of Food Product Packaging (1) – NDI

■Explanation

The various types of commercial food product packaging are designed for ease of use and superior aesthetic design, while at the same time trying to reduce environmental impact and ensure or improve safety. Given these design constraints, the seal portion of packaging is one of the most technically important areas, where ensuring a reliable tight seal is extremely important in terms of safety and quality assurance. Since X-ray CT systems are able to nondestructively observe the internal structure of packaging, it is a popular method of observing the seal status. Therefore, this issue describes several examples of observing the seal area. In this case, an SMX-100CT-SV3 Shimadzu Microfocus X-Ray CT System, which is suitable for objects with relatively low density, was used for observations (see Fig. 10.9.1).

Fig. 10.9.1 SMX-100CT Shimadzu Microfocus X-Ray CT system

■Observation of Plastic Bottle

Fig. 10.9.3 shows CT images of a plastic bottle before and after it was opened (photos of the bottle exterior are shown in Fig. 10.9.2) and Fig. 10.9.4 shows 3D images reconstructed from CT data. These figures show that even after it had been opened once, the bottle was securely sealed (in two locations) when the cap was closed.

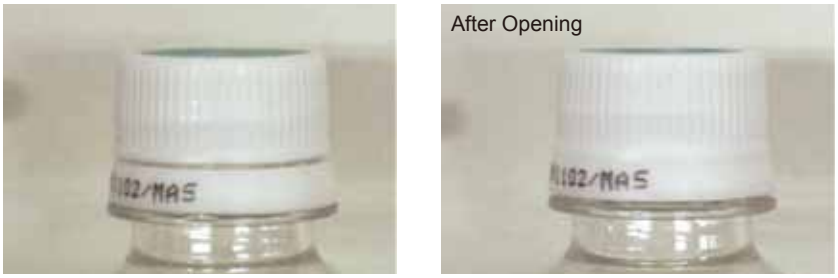


Fig. 10.9.2 Plastic Bottle and Cap

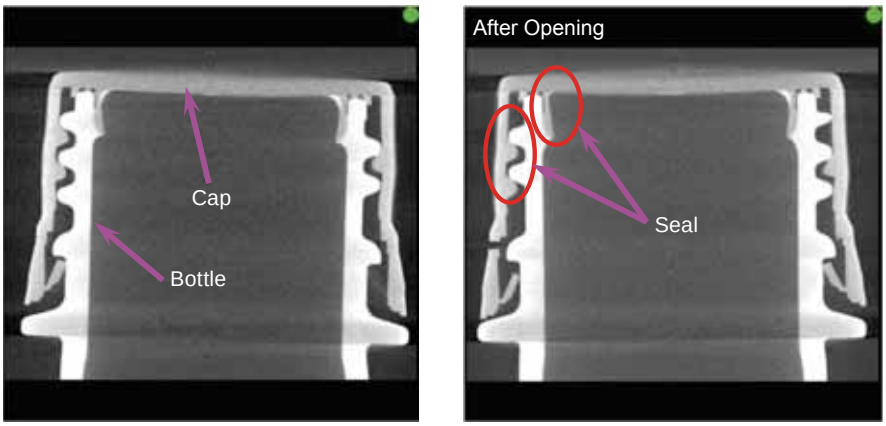


Fig. 10.9.3 CT Image of Plastic Bottle and Cap

10.9 X-Ray CT Observation of Seals of Food Product Packaging (2) – NDI

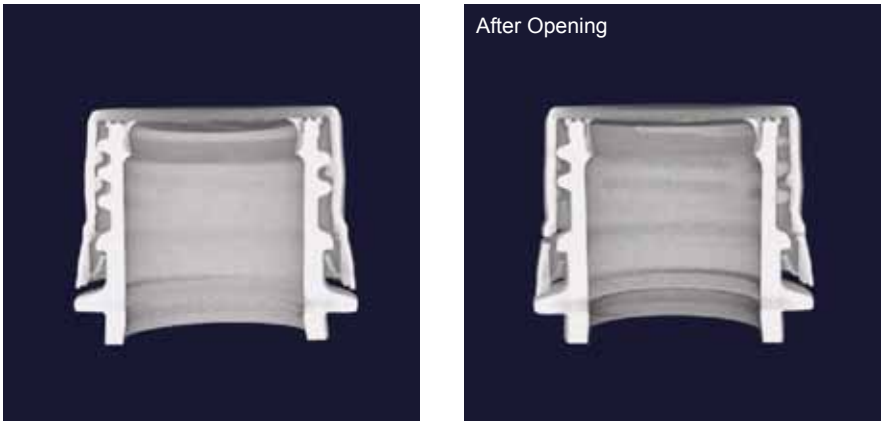


Fig. 10.9.4 3D Image of Plastic Bottle and Cap

■Observation of Food Packaging (1)

As an example of observing packaging seals, CT cross section images and a 3D image of the seal between a cylindrical paper container and its aluminum lid (see diagram in Fig. 10.9.5) are shown in Fig. 10.9.6. These images show that the paper is applied to the outside of container to improve its strength. It also shows that it is securely sealed although the aluminum lid is adhered along the curved surface.

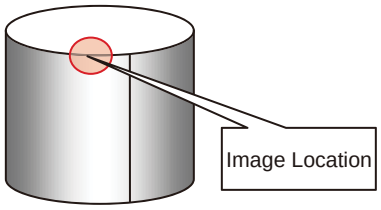


Fig. 10.9.5 Image Location

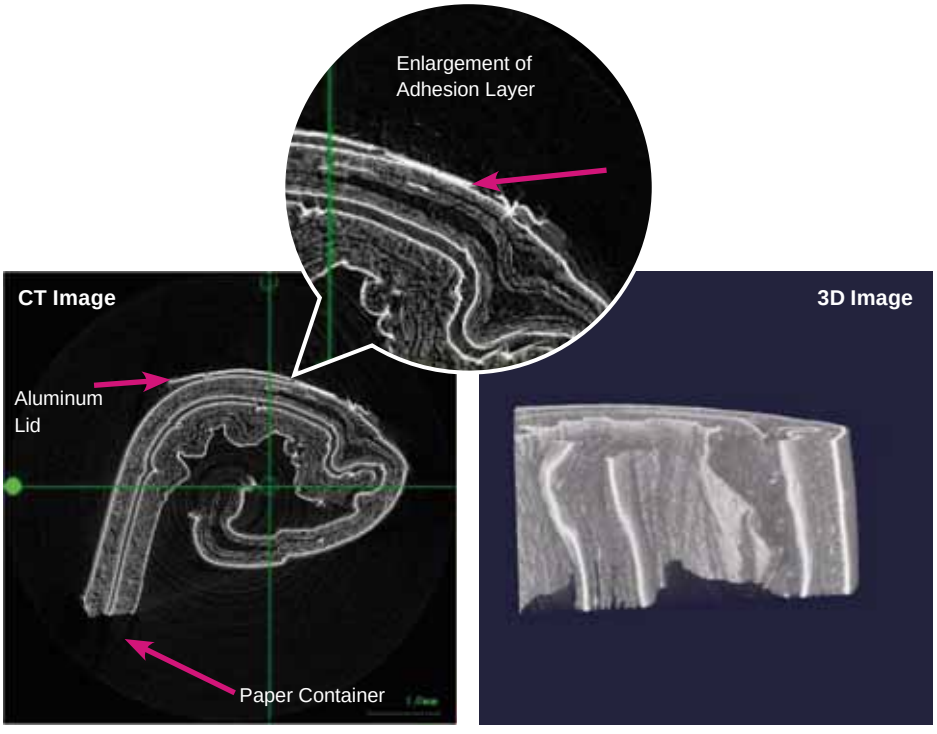


Fig. 10.9.6 Images of Food Packaging

10.9 X-Ray CT Observation of Seals of Food Product Packaging (3) – NDI

■Observation of Food Packaging (2)

In addition, the joint area was observed on two types of containers (cylindrical) similar to the one above (see diagram in Fig. 10.9.7).
The observation example in Fig. 10.9.8 shows how the container is joined by gluing 3 or 4 layers of paper together.

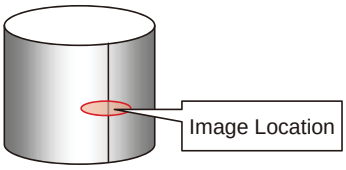


Fig. 10.9.7 Image Location

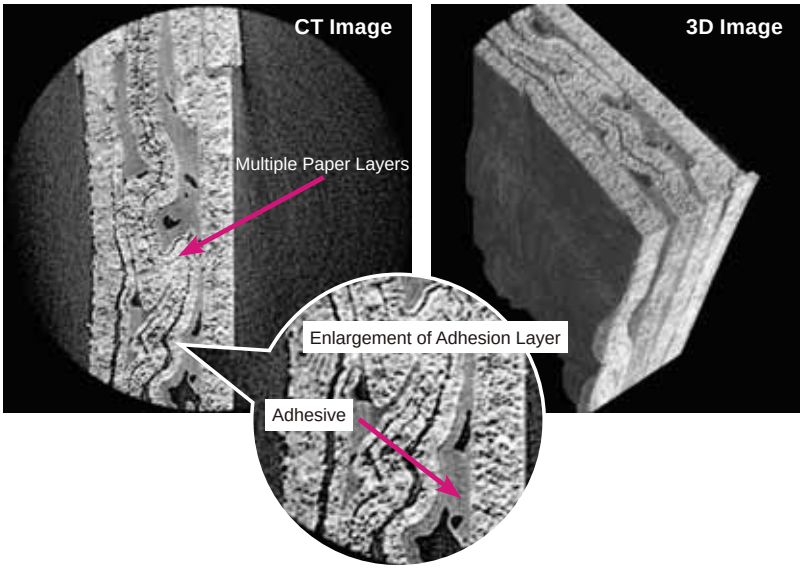


Fig. 10.9.8 Images of Food Packaging

The example in Fig. 10.9.9 is packaging for food products with a high moisture content. The images show in detail how there is aluminum foil between layers of paper and how the container surface is coated with polymer.

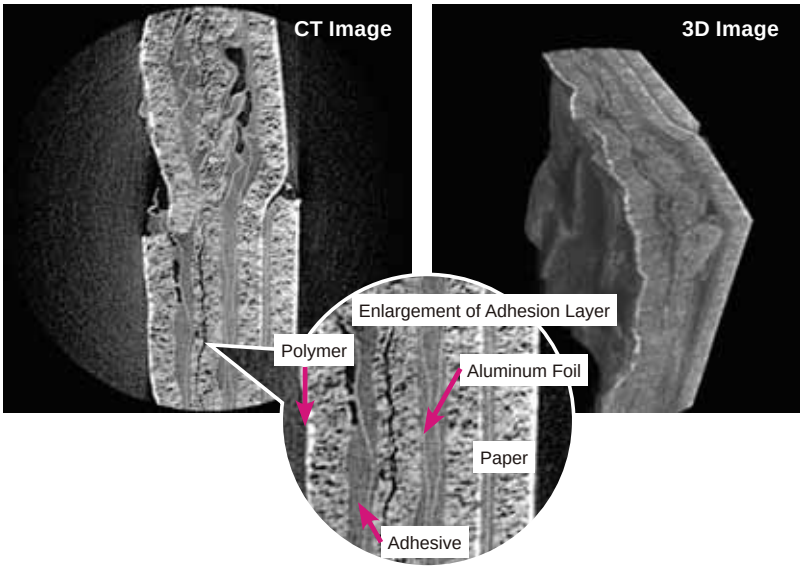


Fig. 10.9.9 Images of Food Packaging

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