Access to your success
SHIMADZU

C297-E086

Microorganism Species Analysis and Component Analysis

– Detection and Identification of Microorganisms and Metabolite Analysis –

Shimadzu's Microorganism Solutions

Microorganism

SHIMADZU BIOTECH
bringing analysis to life

Microorganism Solutions
Analysis

Total Solutions for Microorganism Analysis and Testing

Shimadzu Supports Everybody Working with Microorganisms

While we cannot easily see microorganisms, they are closely linked to our lives. From ancient times, microorganisms have expanded the human diet by providing fermented foods including alcohol and pickles. In recent years, microorganisms have become indispensable in the production of useful substances, such as antibiotics, taste components, and vitamins. Microorganisms are also essential for environmental sustainability and improvements, including wastewater treatment, soil cleanup, and atmospheric balance. Moreover, the application of microorganisms has infinitely extended the possibilities of biotechnologies, which have undergone explosive development since the 1970s. The application of microorganisms also promises solutions to food and energy problems, which are the biggest issues facing mankind.

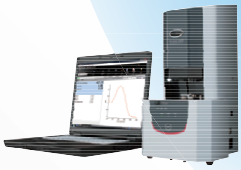
However, microorganisms can also pose a threat to people. For example, *E. coli* O157 bacteria that causes food poisoning, norovirus epidemics, and bacterial contamination of food factories all present serious social problems. In addition, new antibiotic-resistant bacteria such as Methicillin-Resistant *Staphylococcus Aureus* (MRSA) and emerging viruses present significant medical problems. To gain knowledge of microorganisms, diverse analysis technologies are applied to determine not only how many of what bacteria are where (identification and quantitation) but also for research into the response to bacterial components, bacterial-derived components, metabolites, and the environment.

Shimadzu provides an extensive product range, expertise, and solutions to anyone dealing with microorganisms in a diverse range of fields, including medicine, foods, chemicals, energy, the environment, clinical medicine, and hygiene.

Observation and Identification of Microorganisms

DeNOVA-5000HT

AXIMA series



BioSpec-nano

SPM-9600

Detection of Specific Microorganisms

Ampdirect Plus

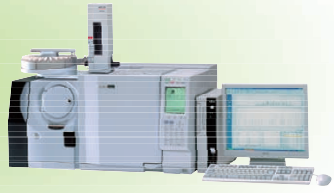
Norovirus G1/G2 detection reagent kit

MultiNA

Basic research to applied research

Prominence UFLC

LCMS-2020



GCMS-QP2010 Plus

Analysis of Microorganism Components

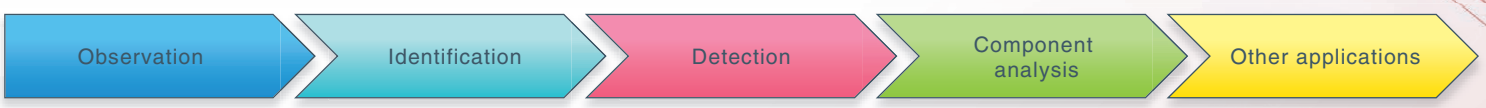
Microorganisms are researched and utilized for a wide range of purposes in many research and industry fields.
The major purposes are inspection and control, identification, searching for new microorganisms, functional investigations, and industrial applications.

Research aims in research and industry fields

Clinical research, hygiene	• Identification and detection
Chemistry	• Inspection and control (pollution countermeasure) • Detection (investigation of biodegradation, etc.) • Extraction of enzymes and other useful components
Energy	• Searching for new microorganisms
Environment	• Functional investigations, functional improvements • Analysis of metabolites • Inspection and control • Recovery of metabolites
Pharmaceuticals	• Searching for new microorganisms • Functional investigations/improvements • Extraction of antibiotics and other useful components • Inspection and control (pollution countermeasures)
Foods	• Fermentation and brewing for food production • Detection (disposition kinetics) • Inspection and control (pollution countermeasures) • Functional investigations • Searching for new microorganisms

Research into microorganisms is multifaceted, from the observation of microorganisms to the chemical analysis of microorganism-derived components.
Consequently, the most important point is the selection of the optimal research approach and the best instruments to support the research. This document introduces examples of the application of Shimadzu products to these research aims.

Workflow for microorganism research



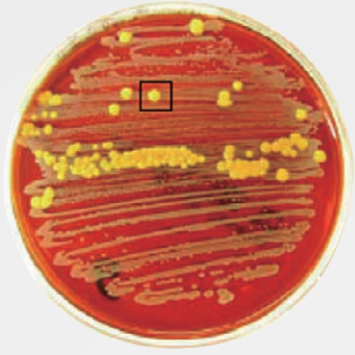
Contents

Microscope Observations of Microorganisms

Cultivation, staining, and microscope observations are widely used for the identification of microorganisms. Generally, observations are performed by optical microscopy combined with staining. However, the detailed morphological features of bacteria and other micron-order microorganisms are difficult to observe at the resolution level of optical microscopes. Shimadzu employs a scanning probe microscope to capture more detailed morphological features of bacteria to better meet the requirements for microorganism observations.

Features of the Scanning Probe Microscope

- The scanning probe microscope permits high-magnification observations in air up to several 10,000x magnification without staining or other pretreatment of the sample surface.
- The electron optical system with excellent low-acceleration voltage characteristics is suitable for shape observations of the sample surface.



Bacteria

Fixing and drying

Scanning Probe Microscope

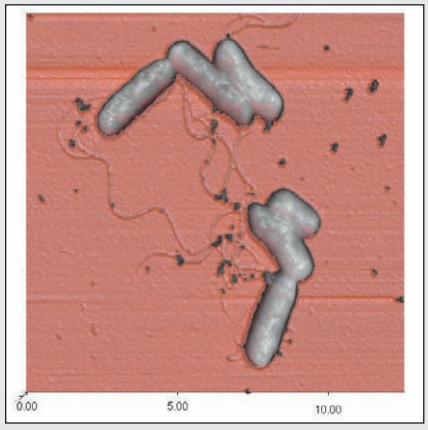
SPM-9600
Scanning Probe Microscope

Data

Scanning Probe Microscope Observations of Microorganisms

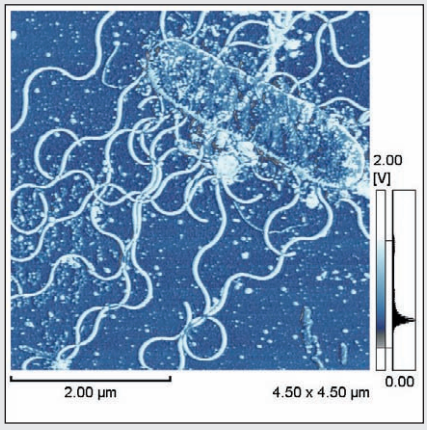
Some scanning probe microscope observations of microorganisms are shown below. The scanning probe microscope offers high-resolution images of microorganisms that cannot be observed by an optical microscope. It requires very simple pretreatment: dripping the bacteria on to a glass sheet, adsorption of the sample, and air-drying only. It clearly captures the detailed morphological features of the bacteria shapes and flagella.

E. coli



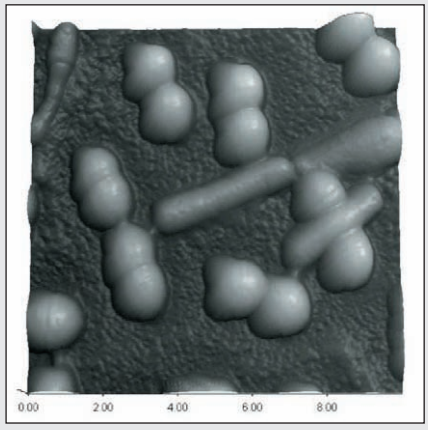
The rod-shaped bacteria and flagella are clearly visible.

Lateral flagella of marine bacteria



This image shows a bacteria culture (*Vibrio* alginolyticus 9) adsorbed on a cover glass in the solution and air-dried. The phase image shows the flagella at high contrast (using a phase detection system). (Courtesy of Dr. Kogure, Ocean Research Institute, University of Tokyo)

Lactobacillus community



This image shows lactobacillus contained in commercially available yoghurt. Two different shapes of lactobacillus are apparent: rod-shaped and spherical bacteria. Particle analysis software can be used for the calculation and statistical processing of the number of lactobacillus with respective morphological features within the field of view to obtain clear morphological images.

The following URL shows many other scanning probe microscope images.
http://www.shimadzu.com/products/lab/surface/spmd.html

Identification of Bacteria by Gene Analysis

The identification of microorganisms by gene analysis has become the general method in recent years. The Japanese Pharmacopoeia shows a method of sequence analysis of part (approx. 300 bases) of bacterial 16S ribosomal DNA (rDNA). However, in practice it may not be possible to clearly distinguish between extremely closely related types using sequence information for only about 300 bases, and multiple types of bacteria can be identified as identical. It is believed that sequence analysis of the entire 16S rDNA (approx. 1,500 bases) is required for accurate identification of bacteria by gene analysis. Sequence decoding with a general DNA sequencer is adequate for a restricted number of samples, such as colonies of specific bacteria produced by separation and cultivation. However, the throughput of a general DNA sequencer is too low to conduct large-scale gene analysis, such as for the currently topical metagenome analysis that identifies microorganisms in entire bacteria colonies. The DeNOVA-5000HT, which allows low-cost, long-chain decoding of multiple samples, is ideal for such analysis.

Features of Microorganism Community Analysis Systems Using the DeNOVA-5000HT High-Throughput BioMEMS DNA Sequencer

- The DeNOVA-5000HT can decode long-chain sequences of 800 bases or more with high statistical reliability (QV > 30 (99.9% reliability)). It covers the entire 16S rDNA (approx. 1,500 bases) with two sequence reactions and decoding from both termini.
- The DeNOVA-5000HT can continuously analyze 384 samples over 24 hours to decode more than 4 Mb per day.
- The DeNOVA-5000HT has an especially high capacity for decoding long chains, which are not handled well by other genome sequencer systems.
- BioSpec-nano is a specialized spectrophotometer for routine laboratory needs for nucleic acid research. It can analyze samples of 1 to 2 µL. To start the analysis, simply drop the sample on to the target and click a button.
- MultiNA uses reusable microchips to achieve automated, high-sensitivity analysis of nucleic acid samples, such as PCR amplified fragments and restriction enzyme digestion fragments. It allows high-performance, automated analysis of up to 120 analyses at 80 sec minimum cycle time.

DeNOVA-5000HT
High-Throughput BioMEMS DNA Sequencer

Decoded sequence information

Database search
(BLAST search)

- Microorganism sequence database search
- Sequence homology search
- Multiple alignment analysis
- Creation of genealogical tree

BioSpec-nano
Life Science Spectrophotometer

MCE-202 MultiNA
Microchip Electrophoresis System
for DNA/RNA Analysis

MultiNA Microchips and Reagent Kits

Data

Gene Analysis of Changes in Microorganism Communities During Water Treatment

The treatment of factory effluent has been attracting greater attention in recent years as one approach to corporate environmental management. Shimadzu installed and operates a wastewater treatment system that uses aeration. The example below shows the evaluation of changes in bacteria flora detected before and after the treatment process.

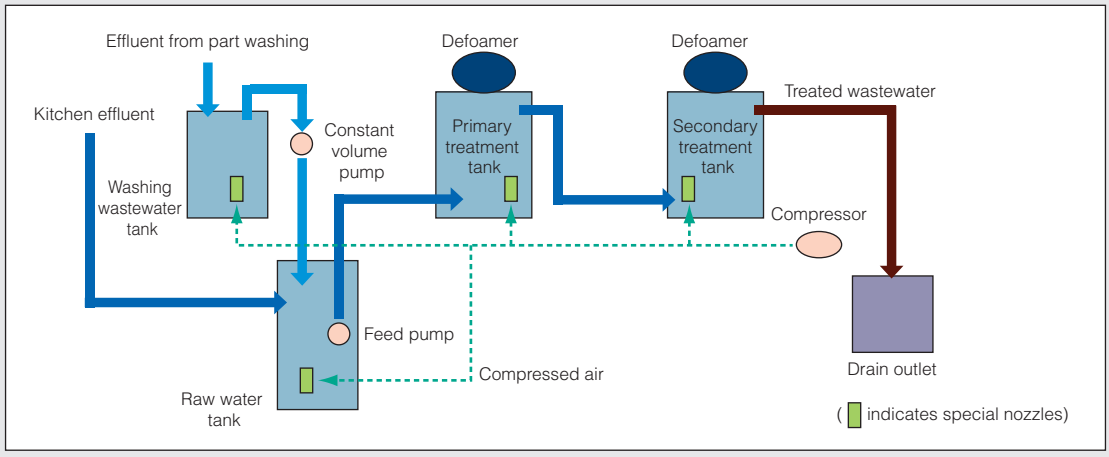


Fig. 1 Schematic Diagram of Wastewater Treatment System

Test and research flow

1. Take constant-volume wastewater samples at each stage before, during, and after treatment and extract the DNA from microorganisms in wastewater.
2. Amplify the 16S rDNA gene by PCR. Create a 16S rDNA library by gene cloning.
3. Conduct qualification and quantitation of the PCR amplification products using MultiNA or BioSpec-nano.
4. Use a DNA sequencer to decode the entire base sequence of the 16S rDNA gene (approx. 1,500 bases).
5. Conduct BLAST searches based on the sequence data and verify against the bacteria 16S rDNA gene sequence database to identify the bacteria type by sequence homology.

Table 1 shows the bacteria types detected or identified in wastewater before, during, and after treatment.
Multiple types of bacteria were detected in the wastewater environment, including several known environmental bacteria.
Gene analysis can easily capture changes in bacteria communities, such as significant increases in species during water treatment by aeration.

Table 1 Number of Bacteria Detected in Wastewater Before, During, and After Water Treatment

	Treatment		
BLAST Top HIT	Before	During	After
Ideonella sp.	6	8	3
Sphingomonas sp.	6	5	4
Rhodobacter sp.	4	4	2
Asticcacaulis sp.	2	5	6
Sinorhizobium	2	3	4
Brevundimonas sp.	2	0	3
Diaphorobacter nitroreducens	0	4	1
Leadbetterella sp.	0	0	3
Brevundimonas diminuta	0	0	2
Uncultured Bacterium PSB-M-1	0	2	0
Uncultured Bacterium A0640	0	2	0
Uncultured Bacterium 054 E02 B DI P58	2	0	0
Uncultured Bacterium LP B54	4	3	0
Uncultured Bacterium HP1B26	3	2	0
Uncultured Bacterium	17	18	21
Other species	9	5	11

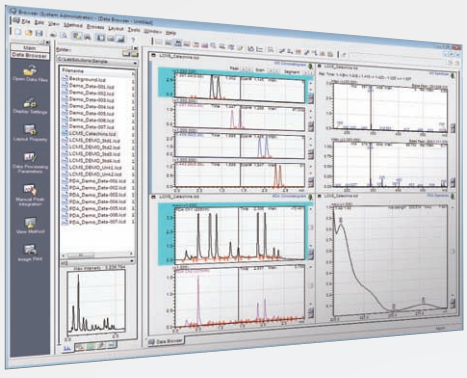
Microorganism Classification by Chemical Analysis

In addition to gene analysis or cultivation, staining, and shape observation, microorganisms can also be classified by the chemical analysis of the microorganism components. Methods previously known for the classification of microorganisms include using the guanine-cytosine content of the constituent DNA as an index for the microorganism genome and the GC or GC/MS analysis of the fatty acid cell membrane components after extraction and derivatization.
LC/MS is a powerful tool for the analysis and taxonomic evaluation of compounds such as isoprenoid quinones with multiple similar structures.

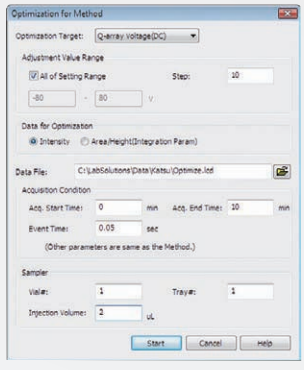
Features of the LCMS-2020 Ultra Fast Liquid Chromatograph Mass Spectrometer

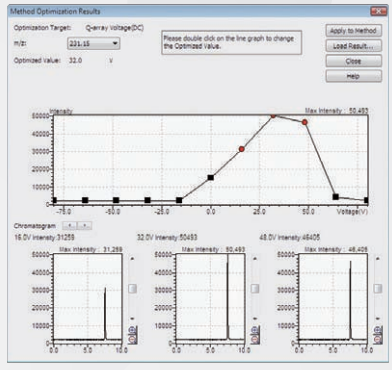
- Rapid 15 msec positive/negative ion switching time permits high-speed detection (MS measurements) that follows high-speed LC separation.
- The newly developed Qarray ion optical system achieves superior sensitivity, reproducibility, and linearity.
- Rapid, 15,000 u/sec scanning achieves high ion permeability while maintaining resolution.
- The LCMS-2020 dramatically enhances long-term stability and ease of maintenance.
- LCMSsolution maximizes analysis performance through comprehensive functions including data comparisons, peak integration, and report output.

LCMS-2020
Ultra Fast Liquid Chromatograph Mass Spectrometer



Browser screen





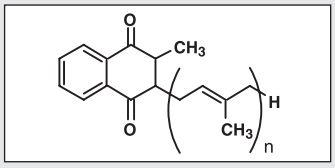
Analytical conditions optimization screens

LCMSsolution Ver.5
LCMS-2020 Workstation

Data

Analysis of the Actinomycete Menaquinone Using LC/MS

The analysis of the bacterial fatty acid composition and isoprenoid quinones has conventionally been used as a chemical classification indicator for actinomycetes.
Typical menaquinone molecule species have been widely researched and provide an extremely effective method for classification and identification at the genus level. This composition nomenclature is essential for naming a new species. LC/MS is a powerful tool for the identification and determination of the composition ratio of menaquinone molecule species.



MK-n (Hm)
····· Expression (1)

[n: number of isoprene units
m: number of hydrogen atoms related to saturation of the isoprene side chains]

Fig. 1 Chemical Structural Formula of Menaquinone

Test and research flow

1. Pulverize freeze-dried bacteria (100 to 300 mg) and extract the lipid components using chloroform/methanol (2:1).
2. Re-dissolve the filtrate in acetone, and roughly separate the menaquinones using TLC silica gel.
3. Re-extract and re-concentrate the menaquinones and perform LC/MS analysis (in ESI-positive mode).

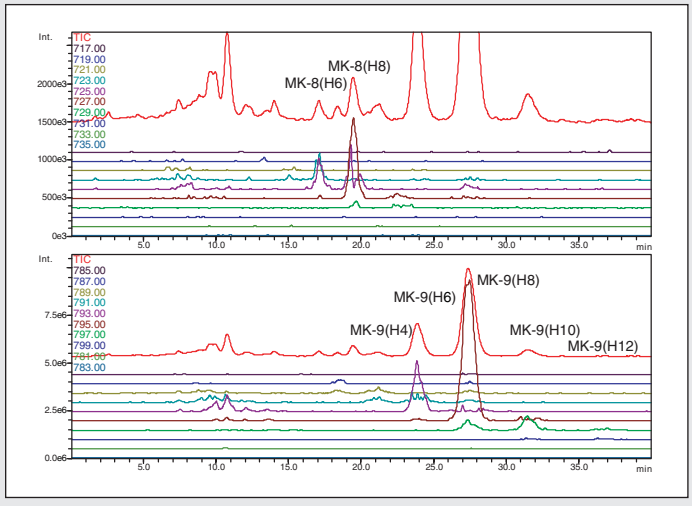


Fig. 2 Total Ion Chromatogram and Mass Chromatogram of Menaquinones

Analytical Conditions

Column	: Shim-pack FC-ODS
Mobile phase	: acetonitrile / methanol / 10 mM ammonium acetate = 2 / 6 / 15
Flow rate	: 0.2 mL/min
Column temp.	: 40 °C
Injection volume	: 6 µL
Probe voltage	: 4.5 kV (ESI-positive mode)
CDL temp.	: 300 °C
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 0.15 MPa
CDL voltage	: 25 V
Qarray DC voltage	: Scan mode
Qarray RF voltage	: Scan mode
Scan range	: *m/z* 200-400 (1.0 sec/scan)
SIM	: *m/z* 331.1, 329.1, 315.1, 313.0 (0.25 sec/ch)

Specific menaquinone molecule species are detected for each actinomycete (Fig. 2 and Table 1). Analysis based on such a chemical classification index is important for the classification and identification of bacteria. LC/MS is an essential analysis tool for this purpose.

Table 1 Menaquinone Molecule Species Content and Relative Retention Time based on MK-6 (H0) for Typical Actinomycete Species

Actinomycetes		**MK-7**	**MK-8**		**MK-9**					**MK-10**			
	JCM No.	**H0**	**H0**	**H2**	**H0**	**H2**	**H4**	**H6**	**H8**	**H0**	**H2**	**H4**	**H6**
Thermoactinomyces candidus	3180T	1.00	1.50										
Rhodococcus equi	1311T			1.77									
Amycolata autotrpphica	4348T			2.05									
Nocardia asteroides	6043T				1.86								
Tsukuamurella pauromethabolum	3226T				1.87	2.11	2.38						
Streptosporangium vulgare	3028T						2.47						
Amycolatopsis orientalis sub sp. Orientalis	4600T						2.39	2.70	3.07				
Actinomadura madurea	7436T							2.66	3.04				
Streptomyces albus subsp. Albus	4450T							2.67	3.05				
Kitasatosporia setae	3304T									2.53	2.91	3.36	3.81
Nocardiopsis assonvillei subsp. Dassonvillei	7437T						2.39					3.32	
Saccharothrix australiensis	3370T												

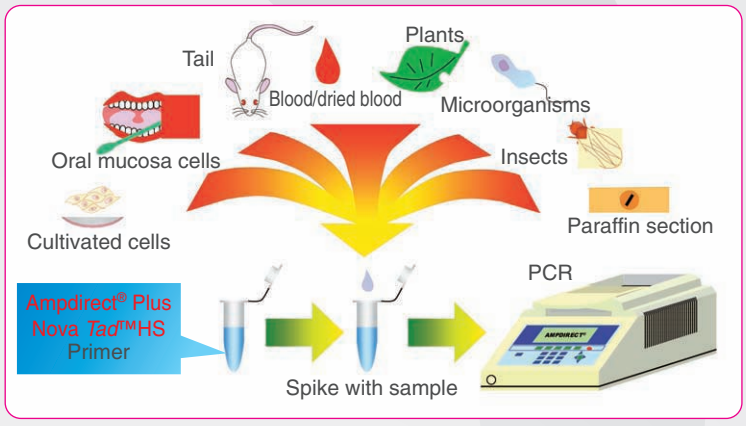
Detection of Microorganism Genes Using Ampdirect Plus

The rapid checking and detection of specific microorganisms, such as bacteria that cause food poisoning, pathogenic microorganisms, and infectious viruses, is demanded in the fields of foods, pharmaceuticals, and the environment. Conventionally, the mainstream testing methods were cultivation testing and methods using the antigen-antibody reaction. Cultivation testing takes a long time and has problems handling microorganisms (such as viruses) that are not easy to cultivate. In recent years, gene-based testing methods have become more prevalent.
Normally, the PCR amplification of genes requires the extraction and purification of template DNA. However, the revolutionary Ampdirect Plus DNA Amplification Reagent developed by Shimadzu eliminates or simplifies this DNA extraction and purification process to accelerate, simplify, and reduce the cost of gene detection.

Features of the Ampdirect Plus Microorganism Gene Detection System

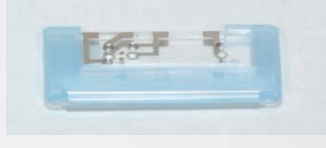
- Ampdirect Plus eliminates the need for (or simplifies) the extraction and purification of template DNA that is required for PCR amplification.
- It reduces inhibition of PCR amplification by proteins, polysaccharides, and other impurities in the sample to achieve stable gene amplification.
- It saves the cost and time required for the extraction and purification of template DNA and reduces the risk of cross-contamination.
- It can detect microorganisms in all samples, including foods, blood (whole blood, plasma, and serum), urine, sputum, throat swab, and feces.
- Combination with the MCE-202 MultiNA Microchip Electrophoresis System for DNA/RNA analysis can automate DNA analysis by agarose gel electrophoresis after PCR amplification.

Ampdirect Plus
DNA Amplification Reagent



Ampdirect Plus offers rapid gene amplification of samples derived from various sources.

MCE-202 MultiNA
Microchip Electrophoresis
System for DNA/RNA Analysis



MultiNA Microchips and Reagent Kits

Data

Detection of Verotoxin-Producing *Escherichia coli* (O157)

According to the "Hygiene Control Manual for Commercial Kitchens" notified by the Japanese Ministry of Health, Labour and Welfare, cooking staff in commercial kitchens producing more than 300 meals of the same menu or more than 750 meals per day must undergo health checks and a stool examination, including a test for enterohemorrhagic *E. coli*, at least once per month and a norovirus examination between October and March, if necessary.

Test flow

1. Take 500 µL 10% fecal suspension (containing *E. coli* O157 10^{-1} to 10^{5} CFU/µL) in a microcentrifuge tube.
2. Heat treat at 95 °C for 5 minutes and centrifuge for 5 minutes to precipitate out the solids.
3. Perform real-time PCR or normal PCR using 1 µL supernatant as the template to detect the verotoxin gene (VT1/VT2) -derived DNA fragments.

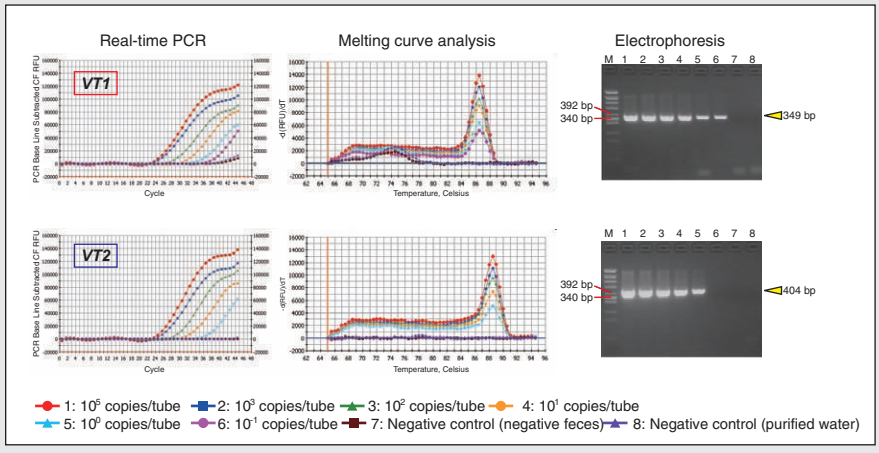


Fig. 1 Real-Time PCR Amplification Curve Using SYBR Green I (Left), Dissociation Curve Analysis (Center), and Verotoxin Gene (VT1/VT2) -Derived DNA Detection Results Using Electrophoresis (Right)

It is apparent that the Ampdirect DNA Amplification Reagent can be used for the high-sensitivity detection of enterohemorrhagic *E. coli*-derived toxin genes in feces (10^{-1} CFU/mL in supernatant, equivalent to 10^{3} CFU/mL in culture fluid).

*CFU : Colony Forming Unit

Detection of Fungi in the Environment

The rapid and simple detection and investigation of microorganisms is demanded due to the recent introduction of HACCP at the manufacturing stage by food manufacturers. The detection of fungus such as mould in the environment using Ampdirect is shown below.

Test flow

1. Spike SDS-Proteinese K solution with a trace amount of bacteria and incubate for one hour at 55 °C.
2. Spike the Ampdirect (PCR) reaction solution with the solution above as the template and perform PCR amplification using a prokaryote/eukaryote common area primer.

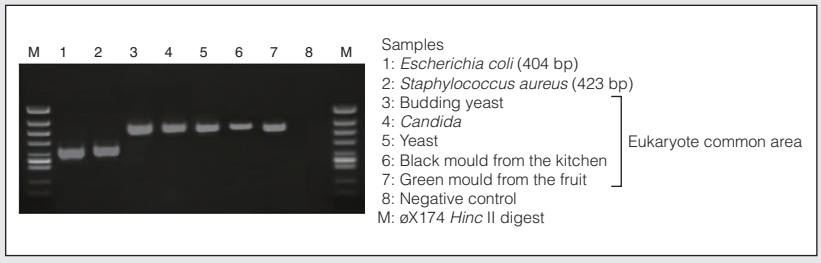


Fig. 1 Results of PCR Amplification Using Ampdirect (Agarose Electrophoresis)

It can be seen that Ampdirect is also effective for the amplification of fungi observed in the environment.
The amplified DNA fragments can be subjected to species identification by sequence decoding or difference distinction based on differences in restriction enzyme fragment lengths (RFLP).

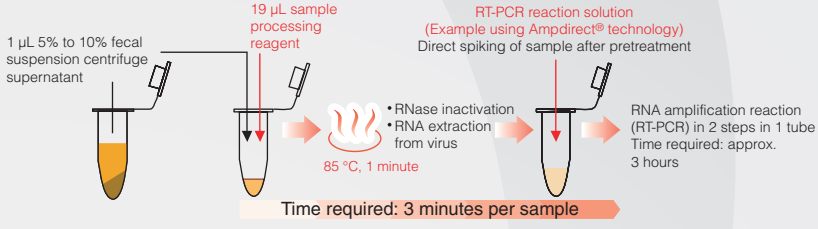
Direct Detection of Norovirus in Feces Using RT-PCR with the Norovirus G1/G2 Detection Reagent Kit

Noroviruses are a cause of food poisoning that can be directly transmitted from the feces or vomit of an infected person, as well as from foods or drinks containing the virus. The 2008 "Hygiene Control Manual for Commercial Kitchens" (No. 0618005 issued by Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare) prescribes a test for norovirus as part of the stool examinations for cooking staff, where required.
Shimadzu developed the Norovirus G1/G2 Detection Reagent Kit to directly amplify and detect two genogroups (G1/G2) from stool samples. It permits direct detection of viruses in feces without the need to purify a template gene.

Features of the Norovirus G1/G2 Detection Reagent Kit

- Simply spike the reagent in the reaction tube; it is not necessary to remove the sample from the reaction tube.
- Eliminates the need for tedious RNA extraction and purification from stool samples and significantly reduces the cost and time required for sample pretreatment.
- Mix centrifuge supernatant from the fecal suspension with the sample processing reagent and incubate for one hour at 85 °C to cause a direct reverse transcriptase reaction and PCR amplification, unaffected by RNA catabolic enzymes or PCR amplification inhibitors.
- All stages from stool sample pretreatment to amplification are performed in a single tube, making the process suitable for processing large volumes.
- The kit reagents are spiked with internal control DNA to prevent false negatives.
- A high correlation has been confirmed with the Japanese Ministry of Health, Labour and Welfare method, which is the standard method for norovirus detection and inspection.
- Combination with the MCE-202 MultiNA Microchip Electrophoresis System for DNA/RNA analysis can automate DNA analysis by agarose gel electrophoresis after PCR amplification.

Norovirus G1/G2 Detection Reagent Kit



The Norovirus Detection Reagent Kit significantly reduces the time for sample pretreatment by eliminating the need for RNA extraction and purification.

MCE-202 MultiNA
Microchip Electrophoresis System for DNA/RNA Analysis

Data

Detection of Norovirus in Feces Using the Norovirus G1/G2 Detection Reagent Kit

Test flow

1. Treat four each of eight stool samples according to the spiking protocol with the G1 and G2 Norovirus Detection Reagent Kit, respectively, and then subject them to the reverse transcriptase reaction and PCR amplification.
2. Detect the amplified genes and evaluate G1/G2 type using the melting temperature (Tm) analysis method by agarose gel electrophoresis or real-time PCR.

G1 \ I.C.		Electrophoresis→Specific amplification product length: 142 bp Tm analysis →Tm peak temperature: 87 °C ± 1 °C	
		Yes	No
Electrophoresis→Specific amplification product length: 86 bp	Yes	Positive	Positive
Tm analysis →Tm peak temperature: 83 °C ± 1 °C	No	Negative	Indeterminate

G2 \ I.C.		Electrophoresis→Specific amplification product length: 205 bp Tm analysis →Tm peak temperature: 89 °C ± 1 °C	
		Yes	No
Electrophoresis→Specific amplification product length: 98 bp	Yes	Positive	Positive
Tm analysis →Tm peak temperature: 85 °C ± 1 °C	No	Negative	Indeterminate

G1/G2 Kit Evaluation Criteria

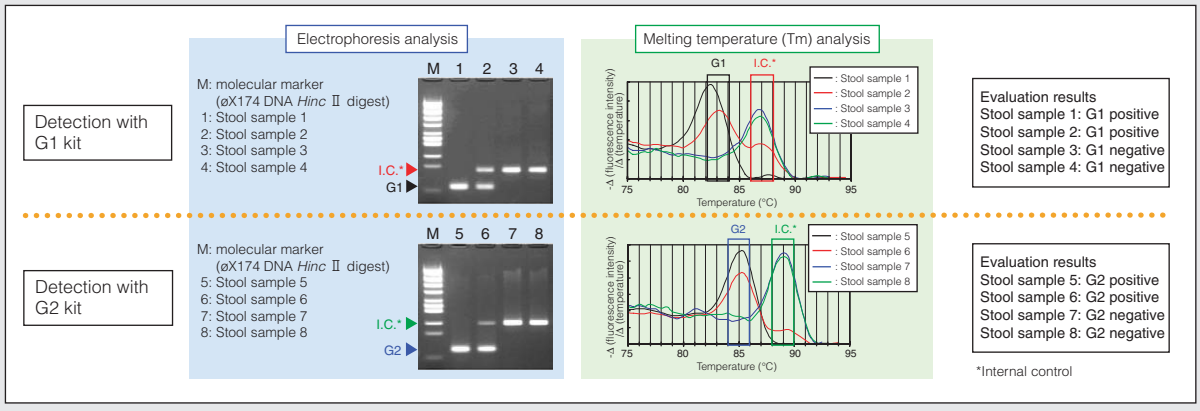


Fig. 1 Detection of Norovirus in Feces Using the Norovirus G1/G2 Detection Reagent Kit
(Agarose Gel Electrophoresis and Melting Temperature Tm Analysis)

Both agarose gel electrophoresis analysis and melting temperature (Tm) analysis were able to clearly detect the absence or presence of norovirus and evaluate the G1/G2 type in all samples.

Detection of Norovirus in Feces Using MultiNA

Both norovirus-derived peaks (86 bp or 98 bp) and peaks derived from the internal control (142 bp or 205 bp) were detected from G1/G2 positive samples.
MultiNA achieves superior separation to agarose gel electrophoresis for PCR amplification fragments up to 100 base pairs.

Fig. 1 shows the results of analyzing amplification products in G1/G2 positive samples using MultiNA with the DNA-500 kit.
MultiNA can perform high-speed, automated analysis of up to 120 samples at equivalent or less cost than agarose gel electrophoresis.

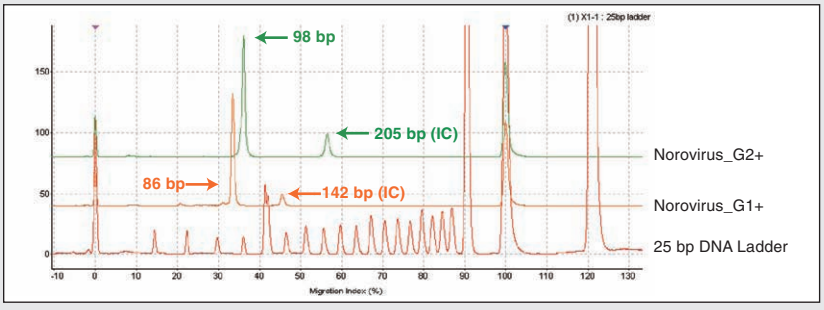


Fig. 1 MultiNA Detection of Norovirus in Feces Using the Norovirus G1/G2 Detection Reagent Kit

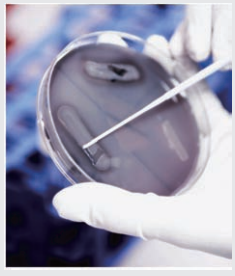
Direct Measurements of Microorganisms Using the MALDI-TOF MS AXIMA Series

The conventional analysis of a microorganism-derived component required the extraction, purification, and analysis of the microorganism (or culture fluid) from which the component is derived. All conventional methods were complex and time-consuming.
In contrast, MALDI-TOF MS can analyze a wider range of compounds and their states.
This feature allows high throughput and low running costs for analysis of microorganism-derived components for the measurement of entire microorganisms.
A new method using MALDI-TOF MS (Intact-Cell MALDI-TOF Mass Spectrometry) is attracting attention for the measurement of entire microorganisms without complex pretreatment.

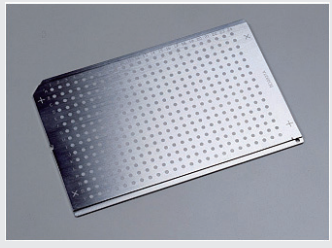
Features of Direct Measurement of Microorganisms Using the MALDI-TOF MS AXIMA Series

- MS analysis possible by simply mixing the microorganism with the matrix solution; no complex sample pretreatment required
- Analysis complete in just two minutes after the start of measurements
- Permits high-throughput analysis (>1,000 samples/day)
- No pretreatment reagent required – low running costs

Sample microorganisms (bacteria)



Apply bacteria sample to sample plate



Mass spectrometry

AXIMA Performance (MS/MS:CID/PSD)
Laser Desorption Ionization Time-of-Flight Mass Spectrometer

AXIMA Confidence (MS/MS:PSD)
Laser Desorption Ionization Time-of-Flight Mass Spectrometer

AXIMA Assurance (MS:Linear)
Laser Desorption Ionization Time-of-Flight Mass Spectrometer

Data

Direct Measurement of Microorganisms Using the MALDI-TOF MS AXIMA Series

Test and research flow

1. Pick up some of the bacteria from a single colony pre-cultivated on the plate.
2. Directly apply the bacteria sample and matrix reagent to the AXIMA MALDI sample plate.
3. Perform mass spectrometry using the MALDI-TOF MS AXIMA Series.

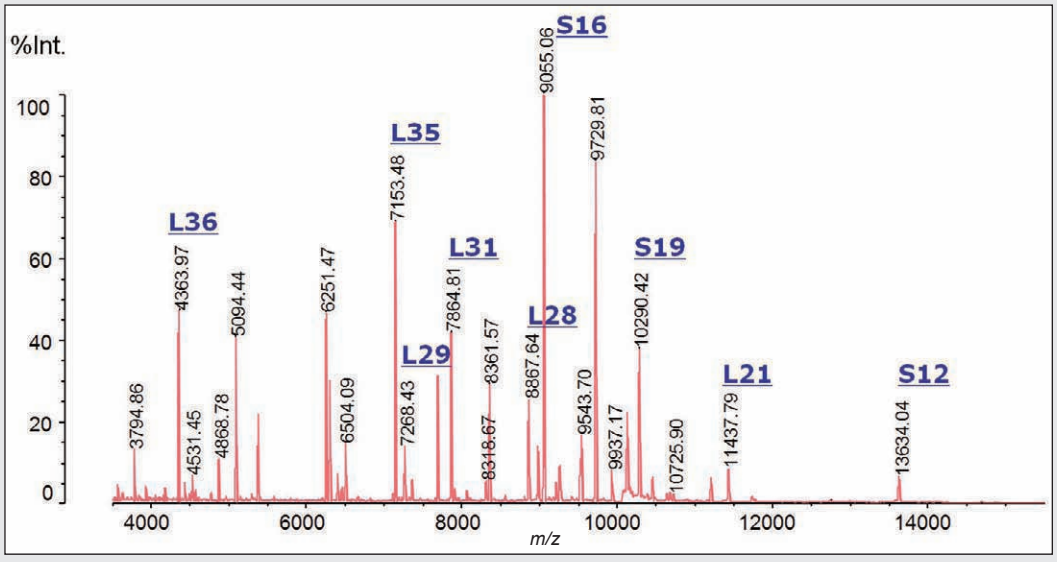


Fig. 1 MS Spectrum of *Escherichia coli* (NBRC 3972 Strain)

Fig. 1 shows the direct measurements of *Escherichia coli* (NBRC 3972 strain). In particular, the ribosomal subunit proteins (Lxx and Sxx peaks on the spectrum) in the bacteria were detected. In addition, multiple ions derived from peptides and lipids can be detected directly from the microorganism without extraction or purification.

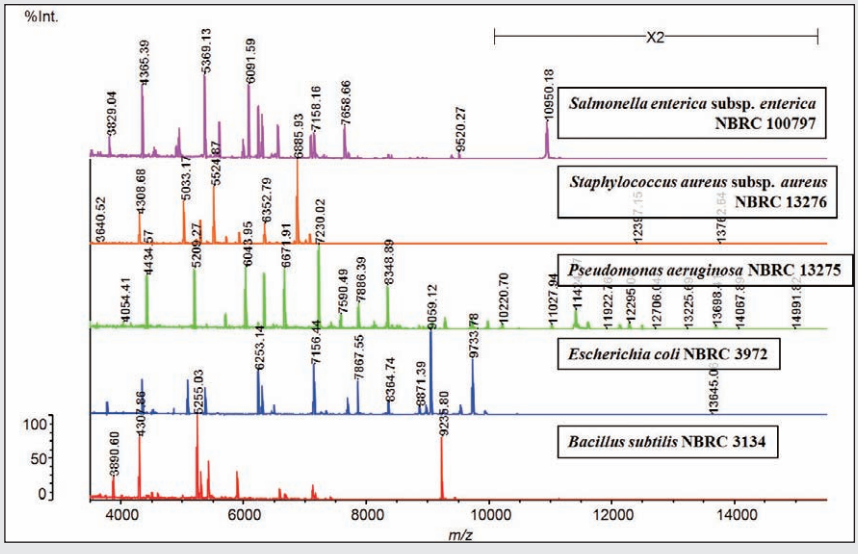


Fig. 2 MS Spectra for Various Bacteria

A comparison of the measurement results for various bacteria samples in Fig. 2 shows that distinctive spectral patterns are obtained according to the bacteria species.

(Data supplied by Prof. K. Tanamoto and Associate Professor M. Muroi, Faculty of Pharmacy, Musashino University and Mr. Y. Nakagawa, National Institute of Technology and Evaluation)

Data

Measurements of Peptides

Fig. 1 shows the MS spectrum of Katanosin B-producing bacteria. *m/z* 1277 is the protonated molecule of Katanosin B. Fig. 2 shows the MS/MS spectrum of Katanosin B.

By measuring directly from the bacteria, MALDI-TOF MS allows simple, high-throughput assays of Katanosin B-producing bacteria.

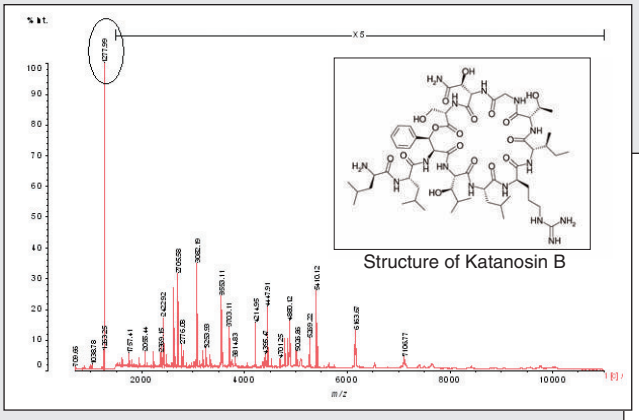


Fig. 1 MS Spectrum of Katanosin B-Producing Bacteria

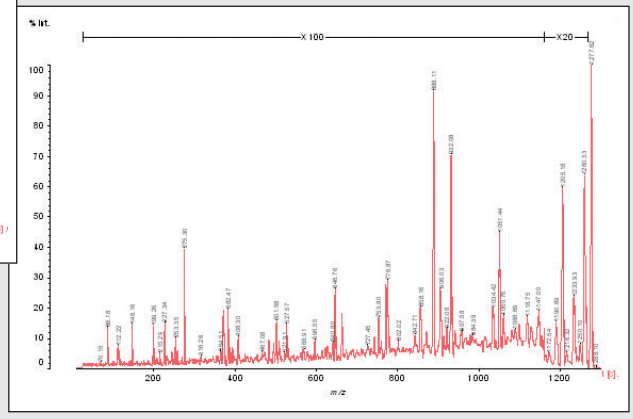


Fig. 2 MS/MS Spectrum of Katanosin B (*m/z* 1277)

Measurement of Lipids

Fig. 1 shows the MS spectrum of Mould A.
Fig. 2 shows the MS/MS spectrum with the *m/z* 804 precursor ion. The *m/z* 804 peak in the MS/MS spectrum is estimated to be phosphatidylcholine (1-acyl-2-acyl 18:2-18:2).

Lipids conventionally analyzed through extraction by organic solvent and analysis by chromatography can be performed by direct measurement of the mould.

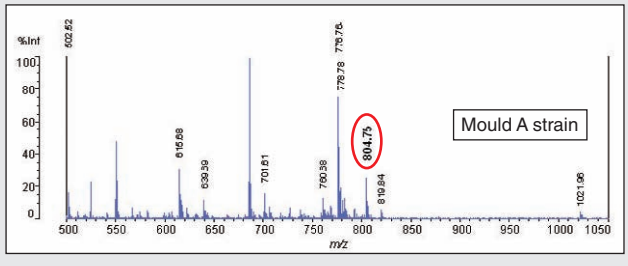


Fig. 1 MS Spectrum of Mould A

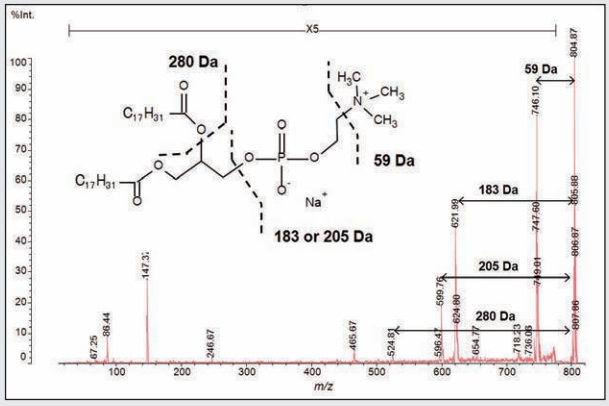


Fig. 2 *m/z* 804 MS/MS Spectrum

(Data supplied by Prof. S. Shimizu and Assistant Professor E. Sakuradani, Graduate School of Agriculture, Kyoto University)

Data

Batch Measurement of Yeast Metabolite Components Using the MALDI-TOF MS AXIMA Series

Test and research flow

1. Pick up some of the sake yeast from a single colony cultivated on the plate.
2. Mix the yeast sample and matrix reagent on the AXIMA MALDI sample plate.
3. Perform mass spectrometry using the MALDI-TOF MS AXIMA Series.

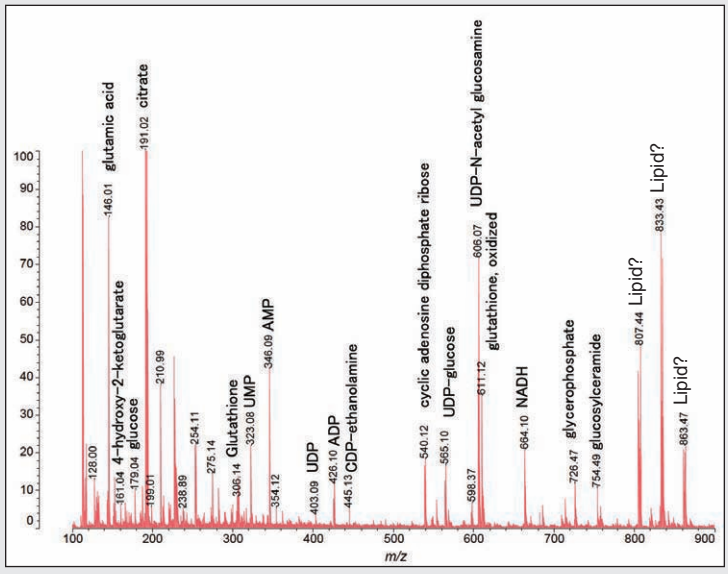


Fig. 1 MS Spectrum of Sake Yeast

In the direct MS measurement of the yeast, many ions derived from biological components, believed to be metabolite components, were observed in the low-mass region (Fig. 1).

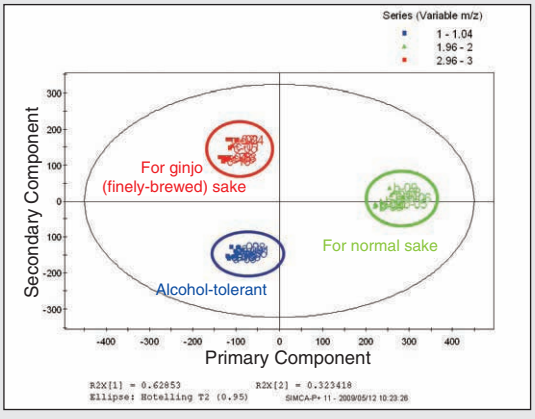


Fig. 3 Primary Component Analysis (PCA)

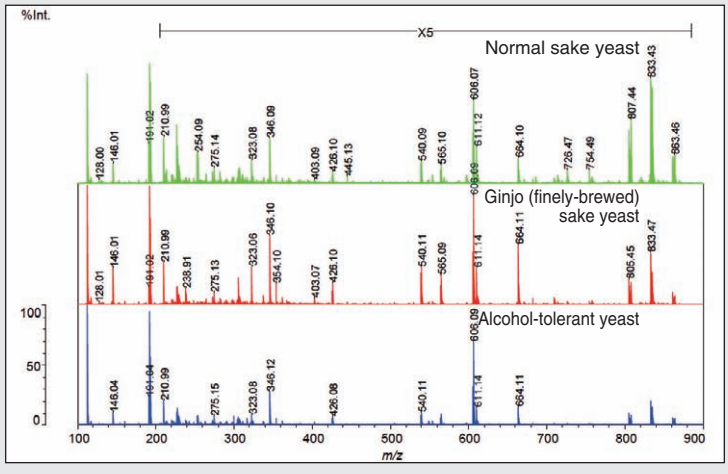


Fig. 2 MS Spectra of Several Types of Sake Yeast

The MS spectra were compared for several types of sake yeast (Fig. 2) and primary component analysis (Fig. 3) was conducted.
The yeast strains were grouped based on the ions detected, and citric acid and glutamic acid were identified as candidate microorganism-derived components to characterize each yeast strain.

(Data supplied by Kizakura Co., Ltd.)

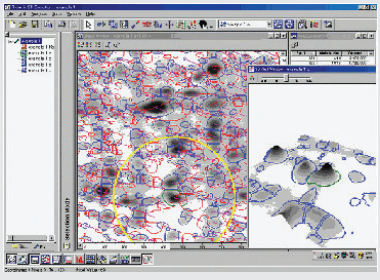
Protein Analysis by MALDI-TOF MS

Peptide mass fingerprinting (PMF) is widely adopted as a proteomics approach for the easy, high-throughput analysis of proteins using MALDI-TOF MS. With this method, after the proteins are subjected to 2D electrophoretic separation, the target proteins are enzyme digested in-gel, and a database search is performed using the mass information for the peptide fragment groups obtained to determine and identify the target proteins.
Direct amino acid sequencing (*de novo* sequencing) using the ORFinder-NB Mass Sequencing Kit (Protein N Terminal Sequencing Kit) and protein determination by definitive amino acid sequencing with a protein sequencer (Edman reaction) and BLAST searches are effective for handling microorganism-derived proteins not included in the protein database.

Features of Protein Analysis (Proteome Analysis) Using the MALDI-TOF MS AXIMA Series

- The Progenesis Electrophoresis Gel Image Analysis Software simplifies quantitative comparisons between electrophoresis gel images and the evaluation of protein spots where a difference is apparent.
- The AXIMA Series offers high-throughput and high-sensitivity protein PMF analysis and MS/MS analysis.
- AXIMA Confidence supports MS/MS (PSD) and AXIMA Performance supports MS/MS (CID/PSD).

Spot matching, qualification and quantitation of changed spots



Progenesis (2D electrophoresis)
Progenesis SameSpots
Electrophoresis Gel Image Analysis Software

Related products

Protein N Terminal Sequencing Kit

ORFinder-NB Mass Sequencing Kit

Mass spectrometry

AXIMA Performance
(MS/MS:CID/PSD)
Laser Desorption Ionization
Time-of-Flight Mass Spectrometer

AXIMA Confidence
(MS/MS:PSD)
Laser Desorption Ionization
Time-of-Flight Mass Spectrometer

Database analysis

MASCOT (Matrix Science)
Mascot Database Search Software (manufactured by Matrix Science Ltd.)

Related products

PPSQ-31A/33A
Protein Sequencing System

Data

Analysis of Protein Extract from *E. coli* by MALDI-TOF MS

Test flow

1. Extract proteins from the *E. coli* culture fluid by the normal method.
2. Perform 2D separation of the proteins using a 2D electrophoresis system (CoolPhoreStar).
3. Analyze the gel images with Progenesis Electrophoresis Gel Image Analysis Software and excise the target protein spots from the gel.
4. Perform in-gel digestion of the target proteins with trypsin. Extract the obtained peptide fragments from the gel and desalt.
5. Perform mass spectrometry using the MALDI-TOF MS AXIMA Series and then perform Mascot database searches (PMF analysis) using the mass list obtained.

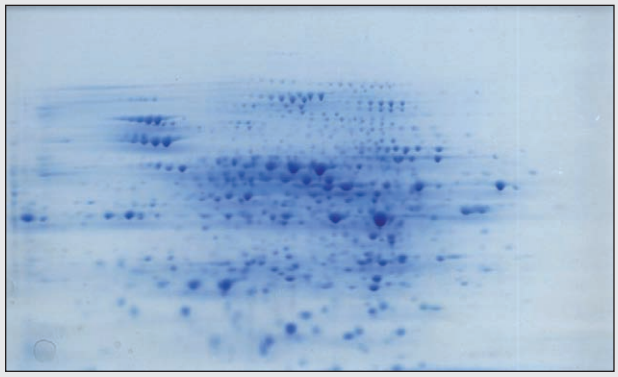


Fig. 1 2D Electrophoresis Image of Protein Extract from *E. coli*

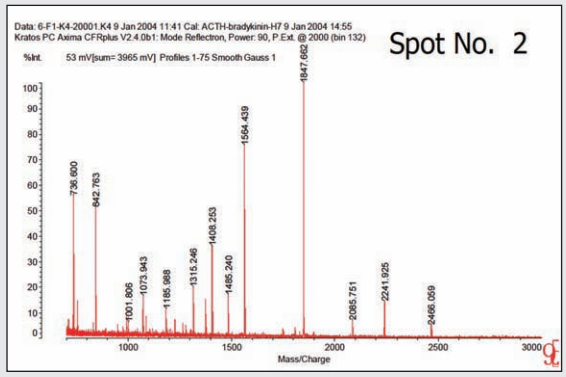


Fig. 2 Mass Spectrum of Spot Arbitrarily Selected from the Gel

Table 1 *E. coli*-Derived Protein Extract Hits by PMF Method (Partial)

Spot No.	Swiss Acc	Mw	pI	Score	Coverage(%)	Protein
1	P16659	63751	5.12	116	26	PROLYL-TRNA SYNTHETASE (EC 6.1.1.15)
2	P17169	66950	5.56	64	16	GLUCOSAMINE--FRUCTOSE-6-PHOSPHATE AMINOTRANSFERASE
3	P17963	34985	4.8	129	38	ADP-L-GLYCERO-D-MANNO-HEPTOSE-6-EPIMERA
5	P10100	37733	5.52	55	12	RARE LIPOPROTEIN A PRECURSOR
7	P39311	22071	4.85	51	20	FKBP-TYPE 22 KDA PEPTIDYL-PROLYL CIS-TRANS ISOMERASE (EC 5.2.1.8)
8	P58065	32682	4.98	78	27	HYPOTHETICAL PROTEIN YNIA
9	P77563	20486	6.75	50	22	APO-CITRATE LYASE PHOSPHORIBOSYL-DEPHOSPHO-COA TRANSFERASE
10	P18197	29579	5.25	88	23	SEPTUM SITE-DETERMINING PROTEIN MIND (CELL DIVISION INHIBITOR MIND).
11	P33667	41484	5.93	45	14	HYPOTHETICAL PROTEIN YBBB
15	P03948	30045	5.56	99	37	2,3,4,5-TETRAHYDROPYRIDINE-2-CARBOXYLATE N-SUCCINYLTRANSFERASE
17	P29132	27943	5.58	50	16	ENOYL-[ACYL-CARRIER-PROTEIN] REDUCTASE [NADH] (EC 1.3.1.9)
23	P27430	18553	5.72	116	54	DNA PROTECTION DURING STARVATION PROTEIN
24	P30138	27352	4.69	43	16	ADENYLYL TRANSFERASE THIF (EC 2.7.7.-).
28	P32665	39087	4.81	57	9	GLYCEROL DEHYDROGENASE (EC 1.1.1.6) (GLDH).
29	P06138	40299	4.65	75	20	CELL DIVISION PROTEIN FTSZ
31	P28635	29471	5.13	65	19	PUTATIVE LIPOPROTEIN YAEC PRECURSOR
35	P00928	28877	5.31	43	13	TRYPTOPHAN SYNTHASE ALPHA CHAIN (EC 4.2.1.20).
36	P02934	37292	5.99	43	10	OUTER MEMBRANE PROTEIN A PRECURSOR (OUTER MEMBRANE PROTEIN II*).
37	P00547	34115	5.45	73	12	HOMOSERINE KINASE (EC 2.7.1.39) (HK)
38	P09743	26030	5.42	53	18	PURINE NUCLEOSIDE PHOSPHORYLASE
40	P09157	21179	5.58	53	13	SUPEROXIDE DISMUTASE [FE] (EC 1.15.1.1)
41	P09157	21179	5.58	53	13	SUPEROXIDE DISMUTASE [FE] (EC 1.15.1.1)
42	P10177	22441	5.57	54	20	KHG/KDPG ALDOLASE [INCLUDES: 4-HYDROXY-2-OXOGLUTARATE ALDOLASE
44	P27430	18553	5.72	177	61	DNA PROTECTION DURING STARVATION PROTEIN
49	P31057	28390	5.15	44	11	3-METHYL-2-OXOBUTANOATE HYDROXYMETHYLTRANSFERASE (EC 2.1.2.11)
52	P39179	36054	5.18	82	18	UNKNOWN PROTEIN FROM 2D-PAGE (SPOT PR51).
53	P07460	41652	5.37	62	17	SUCCINYL-COA SYNTHETASE BETA CHAIN (EC 6.2.1.5) (SCS-BETA).
54	P13519	37100	5.19	49	17	ROD SHAPE-DETERMINING PROTEIN MREB
58	P31223	42228	7.68	93	22	ACRIFLAVINE RESISTANCE PROTEIN A PRECUR
60	P10904	48532	6.29	51	13	GLYCEROL-3-PHOSPHATE-BINDING PERIPLASMIC PROTEIN PRECURSOR.
63	P23929	15062	5.56	64	19	OSMOTICALLY INDUCIBLE PROTEIN C

Peptide mass fingerprinting (PMF) based on MALDI-TOF mass spectrometry offers highly sensitive, high-throughput protein identification from a single 2D electrophoresis spot. This method can be combined with gel image analysis for application to functional analysis of microorganisms, such as screening of proteins expressed characteristically for specific bacterial strains.

GC/MS Analysis of Microorganism-Derived Components

Shimadzu and the Faculty of Medicine, Shimane University, jointly developed the GC/MS Metabolite Component Database, which contains over 300 metabolites (amino acids, fatty acids, organic acids), using a GCMS-QP2010 Plus Gas Chromatograph-Mass Spectrometer (GC/MS). A combination of the GC/MS Metabolite Component Database and a GC/MS permits the batch analysis of biological metabolite components.

Features of the Biological Component Analysis System Using the Combination of GC/MS and the GC/MS Metabolite Component (Amino Acids, Fatty Acids, Organic Acids) Database

- A system comprised of the GCMS-QP2010 Plus and the GC/MS Metabolite Component Database is ideal for research into biological metabolite components, targeting amino acids, fatty acids, and organic acids.
- The database contains not only GC retention indices, mass spectra, and compound information for over 300 metabolites but also analysis method files and data analysis conditions for the target components. It allows fast and easy detection and identification of biological metabolite components, with fewer investigations of analysis conditions and reduced setup work.
- The EZ:faast™ Reagent Kit* simplifies derivatization pretreatment of samples for amino acid analysis.

* EZ:faast™ is manufactured by Phenomenex Inc.

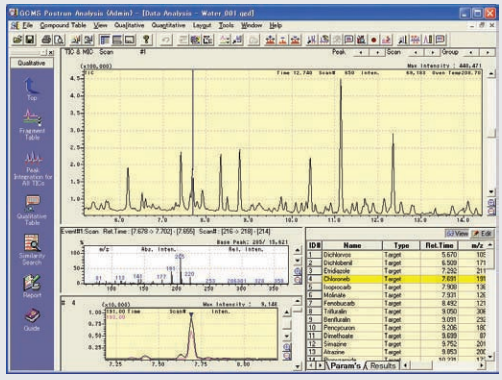
Derivatization

EZ:faast™ kit

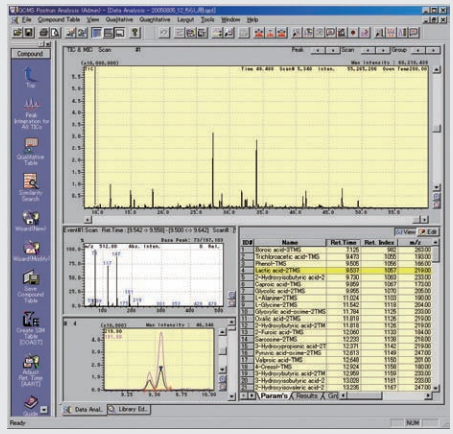
Mass spectrometry

GCMS-QP2010 Plus
Gas Chromatograph - Mass Spectrometer

Data analysis and database research



GCMSsolution Ver. 2 Series
GC/MS Workstation



GC/MS Metabolite Component Database

Data

Fast Analysis of Amino Acids Using the GC/MS Metabolite Component Database

The GC/MS Metabolite Component Database offers optimal column and consumables information, method files containing suitable analytical conditions and compound information, and libraries with retention indices. The metabolite database targets amino acids, fatty acids, and organic acids. It offers four mass spectrum libraries containing retention indices and mass spectra for these derivatives and four method files containing data analysis conditions (Table 1).

EZ:faast™ contains reagents for amino acid analysis pretreatment and derivatization, amino acid standard solution (33 components), pretreatment equipment and an analysis capillary column kit.
Sample pretreatment and derivatization is completed in seven steps (taking approximately seven minutes). In addition to the 33 components above, this kit supports over 50 amino acids and related compounds.

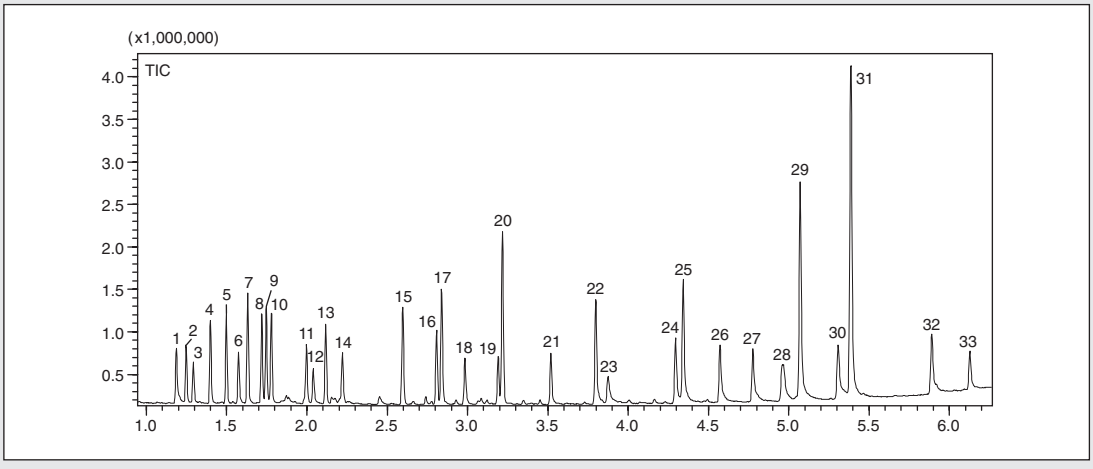
Analytical Conditions

Model	: GCMS-QP2010, GCMS-QP2010 Plus (high-power oven)		
Workstation	: GCMSsolution Ver. 2.5		
Column	: ZB-AAA 10 m × 0.25 mm I.D.		
— GC —		— MS —	
Inj. temp.	: 280 °C	Interface temp.	: 280 °C
Column temp.	: 110 °C (0 min) 30 °C/min	Ion source temp.	: 200 °C
	-320 °C (0 min)	Scan range	: *m/z* : 45-450
Carrier gas	: He	Scan interval	: 0.15 sec
Flow control mode	: pressure		
Pressure	: 15 kPa		
Injection method	: split		
Split ratio	: 1:15		

Table 1 Method Files and Libraries Contained in the Database

Method file/mass spectrum library	Derivatization method	Ionization method	No. of spectra
Amino acid Note)	EZ:faast™	Electron ionization (EI)	33
Fatty acid	Methylation	Electron ionization (EI)	50
Fatty acid	Methylation	Chemical ionization (CI)	50
Organic acid/ amino acid	Trimethylsilylation	Electron ionization (EI)	178

Note) As derivatization methods and libraries using the EZ:faast™ kit require rapid heating rates in the column oven, measurements are performed on a 230 V-specification instrument (high-power oven).



Total Ion Chromatogram of 33-Component Amino Acid Standard Solution

Estimated and Actual Retention Times for 33 Amino Acid Components

ID	Name	R.I.	Retention time			ID	Name	R.I.	Retention time		
			Estimated value	Actual value	Difference				Estimated value	Actual value	Difference
1	Alanine	1675	1.189	1.187	-0.002	18	4-Hydroxyproline	2249	2.923	2.929	0.006
2	Sarcosine	1697	1.249	1.248	-0.001	19	Glutamic acid	2344	3.193	3.191	-0.002
3	Glycine	1712	1.294	1.293	-0.001	20	Phenylalanine	2352	3.215	3.217	0.002
4	alpha-aminobutyric acid	1748	1.401	1.4	-0.001	21	alpha-Aminoadipic acid	2463	3.518	3.519	0.001
5	Valine	1781	1.5	1.498	-0.002	22	alpha-Aminopimelic acid	2569	3.797	3.798	0.001
6	beta-Aminoisobutyric acid	1806	1.575	1.574	-0.001	23	Glutamine	2598	3.872	3.875	0.003
7	Norvaline	1825	1.634	1.631	-0.003	24	Ornithine	2767	4.292	4.295	0.003
8	Leucine	1853	1.721	1.718	-0.003	25	Glycine-proline (dipeptide)	2785	4.335	4.343	0.008
9	allo-Isoleucine	1862	1.749	1.747	-0.002	26	Lysine	2885	4.57	4.572	0.002
10	Isoleucine	1872	1.78	1.779	-0.001	27	Histidine	2977	4.782	4.777	-0.005
11	Threonine	1943	2	1.998	-0.002	28	Hydroxylysine (2 isomers)	3059	4.965	4.958	-0.007
12	Serine	1956	2.04	2.04	0	29	Tyrosine	3107	5.071	5.072	0.001
13	Proline	1980	2.114	2.117	0.003	30	Proline-hydroxyproline (dipeptide)	3218	5.306	5.307	0.001
14	Asparagine	2014	2.219	2.22	0.001	31	Tryptophan	3252	5.376	5.386	0.01
15	Thiaproline	2137	2.594	2.597	0.003	32	Cystathionine	3509	5.901	5.89	-0.011
16	Aspartic acid	2209	2.807	2.807	0	33	Cystine	3626	6.14	6.128	-0.012
17	Methionine	2219	2.836	2.839	0.003						

LC Analysis of Microorganism-Derived Components

The high-performance liquid chromatograph is widely used for the analysis of microorganism-derived components. Due to the diversity of microorganism-derived components, a variety of detectors and applications are required to analyze them with high sensitivity and high accuracy.
Shimadzu supplies a range of detectors from UV-VIS detectors to mass spectrometers to provide the optimal system.

Features of the Prominence High-Performance Liquid Chromatograph

- The Prominence High-Performance Liquid Chromatograph offers superior functionality and performance than conventional LC instruments, with such features as Web control functions and high-sensitivity detection.
- Fully automated analysis, self-diagnostics and auto-recovery (Expert functions), and Web control functions significantly enhance the analysis productivity compared to conventional instruments.
- Detector sensitivity, linearity, and baseline stability are important to improve the reliability of purity testing. The Prominence HPLC offers high sensitivity, superb linearity, and a stable baseline due to ultra-low-pulsation pumping to provide powerful support for purity testing.
- The LCsolution workstation for Prominence offers comprehensive system management functions, FDA 21 CFR part 11-compatible security, and electronic signatures.
- Select the optimum LC column from the wide range of columns from Shimadzu GLC Ltd.

Prominence Amino Acid Analysis System
(with Fluorescence Detector)

Prominence Reducing Sugar Analysis System
(with Spectrofluorometric Detector)

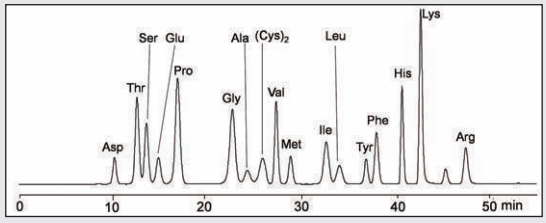
Prominence Analysis System (with ELSD Detector)

Prominence Organic Acid Analysis System
(with Electric Conductivity Detector)

Data

Analysis of Amino Acids (Fluorescence Detector)

The Shimadzu post-column reaction method uses derivatization offering selective reaction with amino bases and spectrofluorometric detection to deliver at least an order of magnitude greater detection sensitivity than the ninhydrin method with UV detection.



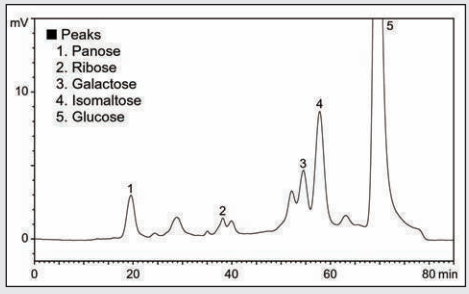
Analysis of Amino Acid Components

Analytical Conditions

Column	: Shim-pack Amino-Na (100 mm L. × 6.0 mm I.D.)
Ammonia trap	: Shim-pack ISC-30/S0504Na (50 mm L. × 4.0 mm I.D.)
Mobile phase	: Amino Acid Mobile Phase Kit (Na type), gradient elution
Flow rate of mobile phase	: 0.4 mL/min
Column temp.	: 60 °C
Reaction reagent	: Amino Acid Reagent Kit
Flow rate of reaction reagent	: 0.2 mL/min, each
Reaction temp.	: 60 °C
Detection	: Ex at 350 nm, Em at 450 nm

Analysis of Saccharides in Brewed Products (Spectrofluorometric Detector)

Due to its selectivity and sensitivity, post-column fluorescence derivatization is suitable for the analysis of saccharides in brewed products.
This is an example of the batch analysis of saccharides in Japanese sake with Shimadzu's Reducing Sugar Analysis System, which uses a unique arginine reagent.



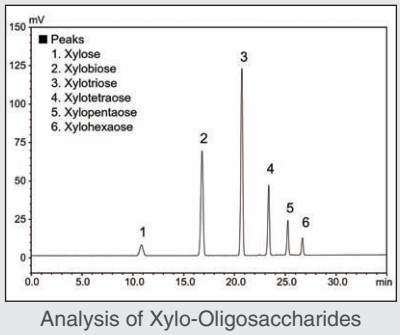
Analysis of Sake

Analytical Conditions

Column	: Shim-pack ISA-07/S2054 (250 mm L. × 4.6 mm I.D.)
Guard column	: ISA (50 mm L. × 4.6 mm I.D.)
Mobile phase	: A: 0.1 M (potassium) borate buffer (pH8) B: 0.4 M (potassium) borate buffer (pH9) A → B / linear gradient elution
Flow rate	: 0.6 mL/min
Column temp.	: 65 °C
Reagent	: 1% arginine, 3% boric acid
Flow rate of reagent	: 0.5 mL/min
Reaction temp.	: 150 °C
Detection	: Ex at 320 nm, Em at 430 nm

Analysis of Saccharides in Bioethanol Production (ELSD Detector)

Bioethanol is produced by fermenting saccharides derived from biomass materials, such as sugar cane or maize.
This is an example of the analysis of saccharides related to bioethanol production using the ELSD-LT II Evaporative Light Scattering Detector.



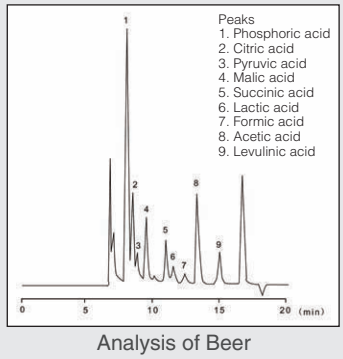
Analysis of Xylo-Oligosaccharides

Analytical Conditions

Column	: Unison UK-Amino (250 mm L. × 4.6 mm I.D., 3 µm)
Mobile phase	: A: water, B: acetonitrile B Conc. 90% (0 min) → 90% (5 min) → 55% (30 min) → 30% (31–36 min) → 90% (36.01–51 min)
Flow rate	: 1.0 mL/min
Column temp.	: 25 °C
Injection volume	: 10 µL
Detection	: ELSD-LT II Temperature : 40 °C Nebulizing gas : N_2 Gain : 6 Gas pressure : 350 kPa

Analysis of Organic Acids (Electric Conductivity Detector)

Absorptiometric detection is used for the analysis of organic acids. However, as the detection wavelength is near the 205 nm peak derived from the absorption of the carboxyl group, this method is susceptible to effects from impurities and some samples require complex pretreatment. In some cases analysis is extremely difficult.
This is an example of the analysis of beer using an electric conductivity detector, which is highly sensitive and selective for ionic substances.



Analysis of Beer

Analytical Conditions

	For separation
Column	: Shim-pack SCR-102H (300 mm L. × 8.0 mm I.D.)
Mobile phase	: 5 mM *p*-toluenesulfonic acid
Flow rate	: 0.8 mL/min
Temperature	: 45 °C
	For detection
Reagent	: 5 mM *p*-toluenesulfonic acid, 20 mM Bis-Tris and 100 µM EDTA
Flow rate	: 0.8 mL/min
Temperature	: 48 °C
Detection	: electric conductivity

UFLC Analysis of Microorganism-Derived Components

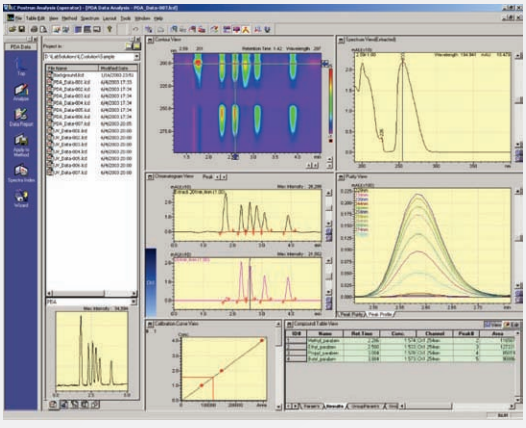
High-performance liquid chromatography is widely used for the analysis of amino acids in fermented and brewed food products. However, the demand for fast analysis has been increasing over recent years to enhance the productivity of laboratory analysis. The Prominence Ultra Fast Liquid Chromatograph (UFLC) is based on a comprehensive analysis of technologies to increase the speed of liquid chromatography. In addition to ultra-fast liquid chromatography, it achieves high levels of accuracy, stability, durability, and expandability not available from conventional HPLC. The ultra-fast analysis and high accuracy and reliability offered by the Prominence UFLC achieve revolutionary improvements in laboratory analysis productivity.

Features of the Prominence UFLC Ultra Fast Liquid Chromatograph

- Ultra-fast LC analysis: just one-tenth the analysis time of general LC
- Shorter total analysis cycle time
- High-resolution analysis: three times the resolution of general LC
- High accuracy, stability, and durability
- The Shim-pack XR-ODS Column for fast analysis and high separation maximizes the Prominence UFLC performance.

Prominence UFLC
ULTRA FAST LIQUID CHROMATOGRAPH

Prominence UFLC

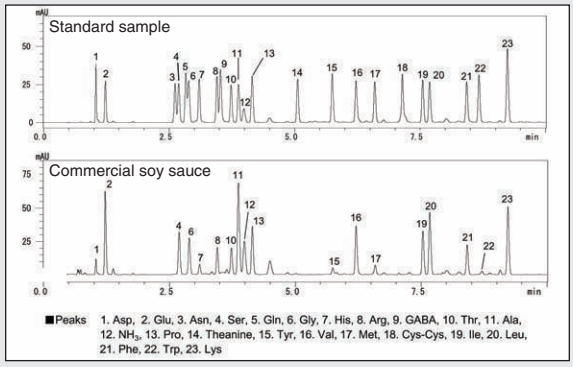


LCsolution Screen

Shim-pack XR-ODS
Column for Fast Analysis and High Separation

Data

Fast Analysis of Amino Acids by Pre-Column Derivatization



Analytical Conditions

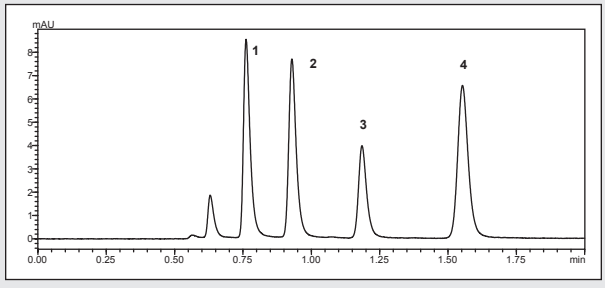
Column	: Shim-pack XR-ODS (100 mm L. × 3.0 mm I.D.)
Mobile phase	: A) 10 mmol/L (potassium) phosphate buffer (pH7.0) B) Acetonitrile B.conc; 5% (0 to 0.5 min), 5% to 35% (0.5 to 10.5 min)
Flow rate	: 0.9 mL/min
Column temp.	: 40 °C
Detection	: absorbance at 254 nm with semi-micro flow cell
Sample volume	: 1 µL

Derivatization Reaction with Phenylisothiocyanate (PITC)

1. To 200 µL sample, add 100 mmol/L phenylisothiocyanate (acetonitrile solution) and 1 mol/L triethylamine (acetonitrile solution), agitate, and leave at 40 °C for 20 minutes to react.
2. Allow to cool to room temperature, and spike with 1.2 mol/L hydrochloric acid aqueous solution to neutralize.

This is an example of the fast analysis of amino acids by pre-column derivatization using PITC. Analysis was performed on amino acids, 23 related components (0.5 µmol/L), and commercially available soy sauce. The analysis was completed within 10 minutes.

Fast Analysis of Organic Acids



The ion exclusion mode is often used for the analysis of organic acids, but in this case an ODS column that can handle 100% aqueous solutions was used to complete fast analysis within two minutes.

Analytical Conditions

Column	: Phenomenex Synergi 2.5 µm Hydro-PP 100A (100 mm L. × 3.0 mm I.D., 2.5 µm)
Mobile phase	: 10 mmol/L (sodium) phosphate buffer (pH2.6)
Flow rate	: 0.8 mL/min
Column temp.	: 30 °C
Injection volume	: 4 µL
Detection	: SPD-20A at 210 nm
Flow cell	: semi-micro cell

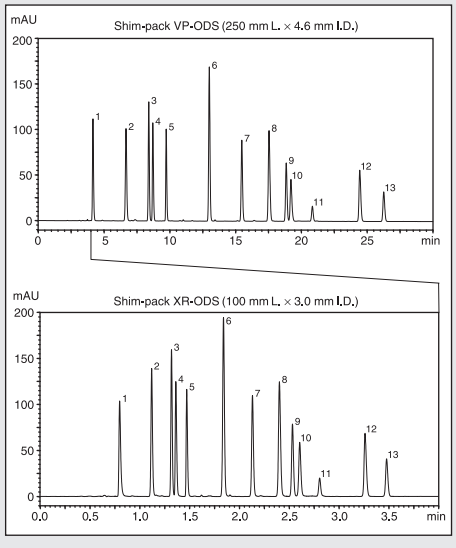
Fast Analysis of Cephem Antibiotics

Analytical Conditions

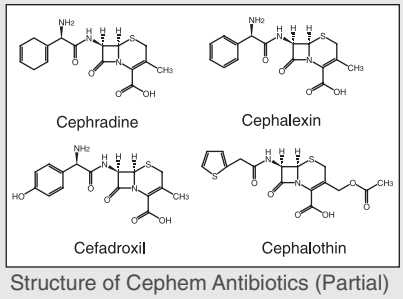
Column	: Shim-pack XR-ODS (100 mm L. × 3.0 mm I.D., 2.2 µm) Shim-pack VP-ODS (250 mm L. × 4.6 mm I.D., 4.6 µm)
Mobile phase	: A:0.1% formic acid-water B:acetonitrile
Time program	[XR-ODS] B.conc; 15% (0 min)→ 55% (3.5 min) → 15% (3.51-6.5 min) [VP-ODS] B.conc; 15% (0 min)→ 55% (30 min) → 15% (30.01-45 min); 0.2 mL/min
Flow rate	: 1.0 mL/min (XR-ODS) 1.0 mL/min (VP-ODS)
Column temp.	: 40 °C
Injection volume	: 4 µL (XR-ODS) 10 µL (VP-ODS)
Detection	: photodiode array UV-VIS detector 260 nm
Flow cell	: semi-micro cell (XR-ODS) conventional (VP-ODS)

■ Peaks

1 Cefadroxil, 2 Cephapirin, 3 Cefaclor
4 Cefalexin, 5 Cephradine, 6 Cefotaxime
7 Cefazolin, 8 Cefuroxime, 9 Cefoperazone
10 Cefoxitin, 11 Cefamandole A, 12 Cephalothin
13 Cefamandole B (50 mg/L each)



Chromatogram of 13 Cephem Antibiotic Components
(Upper: Shim-pack VP-ODS;
Lower: Shim-pack XR-ODS)



Structure of Cephem Antibiotics (Partial)

Cephem antibiotics are a type of beta-lactam antibiotic. They are broad-spectrum antibiotics with a strong antibiotic action, and are used as general-purpose oral or injected antibiotics. The combination of Prominence UFLC and Shim-pack XR-ODS increases the mobile phase linear velocity to approximately 2.4 times that of the Shim-pack VP-ODS during analysis. In addition, it maintains high resolution while reducing the analysis cycle time by approximately 85%.

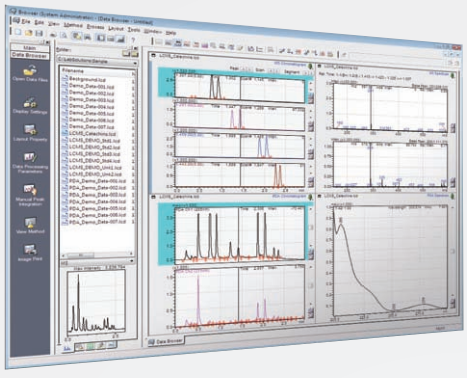
LC/MS Analysis of Microorganism-Derived Components

Liquid chromatography has been widely used for the analysis of microorganism-derived components. However, the UV detector cannot achieve adequate sensitivity for compounds with almost no UV absorption. Sophisticated investigation of the separation conditions is often necessary, such as for the analysis of trace levels of components in a mixture. LC/MS uses a variety of methods to ionize the sample components. This method separates these ions in a vacuum according to the mass-to-charge ratio (*m/z*) and detects the ion intensities at high sensitivity. The mass is information unique to the molecule and directly using this information makes it simple to analyze complex component mixtures.
The Japanese Ministry of Health, Labour and Welfare has set and notified standards and test methods for mycotoxins such as patulin and aflatoxin produced as secondary metabolites of mould.

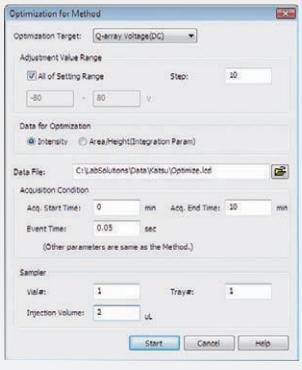
Features of the LCMS-2020 Ultra Fast Liquid Chromatograph Mass Spectrometer

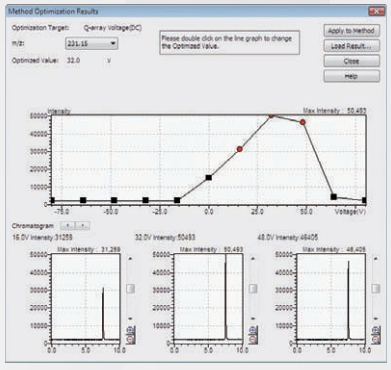
- Prominence UFLC offers both speed and separation capacity as well as high accuracy and expandability not available from conventional HPLC. Used with a high-speed, high-accuracy autosampler, it achieves genuine high throughput.
- Rapid 15 msec positive/negative ion switching time permits high-speed detection (MS measurements) that follows high-speed LC separation.
- The newly developed Qarray ion optical system achieves superior sensitivity, reproducibility, and linearity.
- Rapid, 15,000 u/sec scanning achieves high ion permeability while maintaining resolution.
- The LCMS-2020 dramatically enhances long-term stability and ease of maintenance.
- LCMSsolution maximizes analysis performance with a number of comprehensive functions including data comparisons, peak integration, and report output.

LCMS-2020
Ultra Fast Liquid Chromatograph Mass Spectrometer



Browser screen





Analytical conditions optimization screens

LCMSsolution Ver.5
LCMS-2020 Workstation

Data

Analysis of Aflatoxins

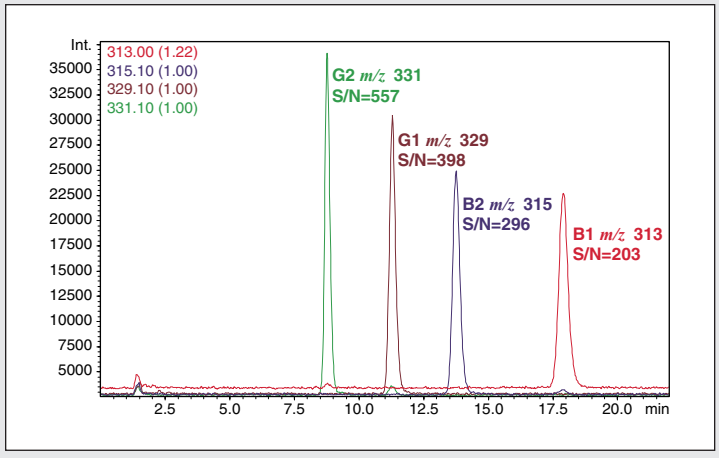


Fig. 1 SIM Chromatogram of Aflatoxin Mixture (2.5 ppb each)

Aflatoxin is a natural, highly carcinogenic toxic substance produced by a mould (*Aspergillus*). This mould grows on crops such as peanuts and corn. The Japanese Ministry of Health, Labour and Welfare notified a test method for aflatoxins in 1971. The Japanese Food Sanitation Act regulates foods in which more than 10 ppb aflatoxin is detected. The aflatoxin test method was upgraded to use non-toxic reagents on 26 March 2002 and new LC fluorescence detection and LC/MS methods were adopted.

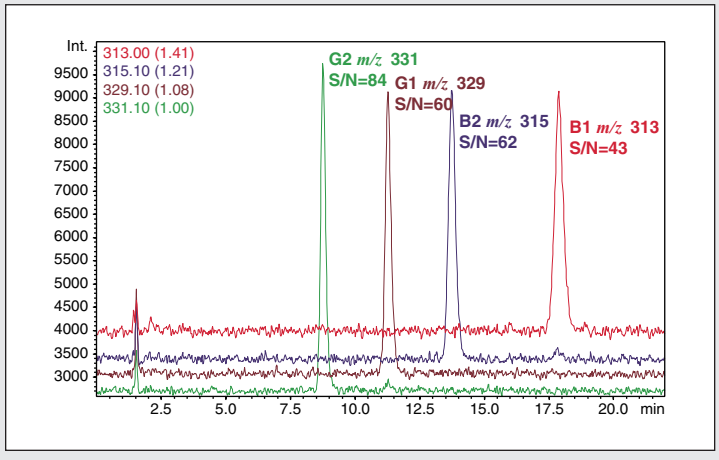


Fig. 2 SIM Chromatogram of Aflatoxin Mixture (0.5 ppb each)

Analytical Conditions

Column	: Imtakt Cadenza CD-C18 (150 mm L. × 2.0 mm I.D.)
Mobile phase	: acetonitrile / methanol / 10 mM ammonium acetate = 2 / 6 / 15
Flow rate	: 0.2 mL/min
Column temp.	: 40 °C
Injection volume	: 6 µL
Probe voltage	: 4.5 kV (ESI-positive mode)
CDL temp.	: 300 °C
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 0.15 MPa
CDL voltage	: 25 V
Qarray DC voltage	: Scan mode
Qarray RF voltage	: Scan mode
Scan range	: *m/z* 200-400 (1.0 sec/scan)
SIM	: *m/z* 331.1, 329.1, 315.1, 313.0 (0.25 sec/ch)

Fig. 1 shows the SIM chromatogram of four aflatoxin components, G2, G1, B2, B1 (each 2.5 ppb), and Fig. 2 shows the SIM chromatogram of 0.5 ppb concentrations. A satisfactory S/N ratio was obtained, even at 0.5 ppb concentration of each component.
The LC/MS test method for aflatoxins conforming to the official analytical method is introduced here.
Fig. 3 shows the MS spectra and calibration curves for aflatoxin G2 (600 pg) and aflatoxin G1 (600 pg). The calibration curves achieve good linearity with r^2 (coefficient of determination) = 0.9999 min.

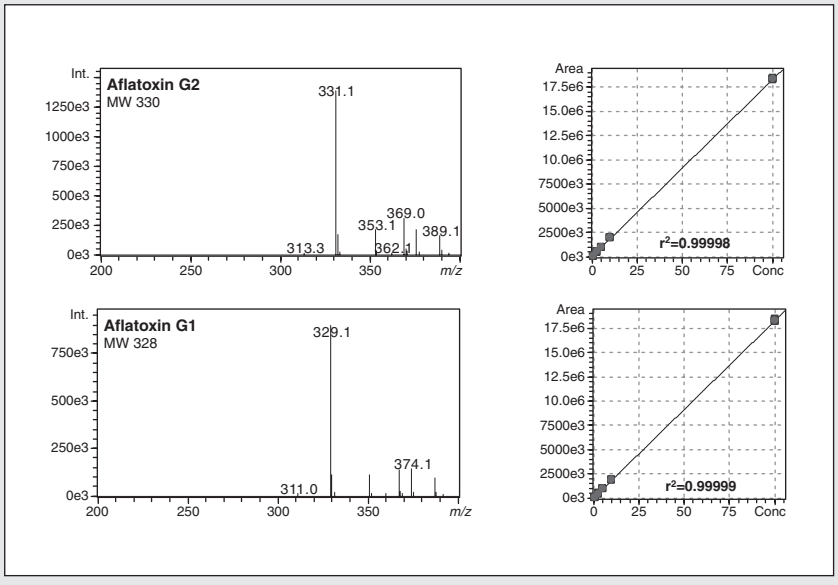


Fig. 3 MS Spectra and Calibration Curves for Aflatoxin G2 and G1

Data

Analysis of Amino Acids

Amino acid is a generic term for compounds that contain an amino group and a carboxyl group. Several hundred of them exist naturally. Amino acids are the basic units comprising proteins, which are one of the major biological components. They provide the materials to synthesize the neurotransmitters and low-molecular-weight bioactive compounds and even alone offer a variety of bioactivities. They are widely researched in the fields of pharmaceuticals and foods and many amino acids have been used for health food supplements in recent years.

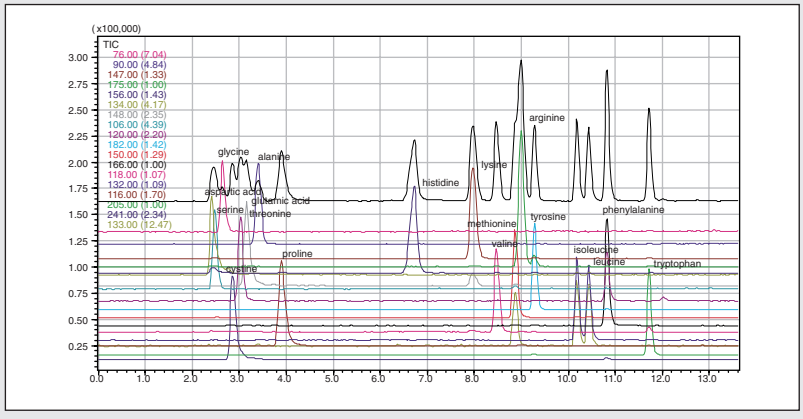


Fig. 1 SIM Chromatogram of a Standard Mixture of 18 Amino Acid Components

Analytical Conditions

Column	: Shim-pack FC-ODS (150 mm L. × 2.0 mm I.D.)
Mobile phase	: ion pair reagent-water / acetonitrile
Time program	: gradient elution
Flow rate	: 0.2 mL/min
Injection volume	: 3 µL
Column temp.	: 40 °C
Probe voltage	: +4.5 kV (ESI-positive mode)
CDL temp.	: 200 °C
Block heater temp.	: 200 °C
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 0.1 MPa
CDL voltage	: +10 V
Qarray DC voltage	: Scan mode
Qarray RF voltage	: Scan mode
SIM	: *m/z* 76, 90, 147, 175, 156, 134, 148, 106, 120, 182, 122, 150, 166, 118, 132, 116, 205, 241

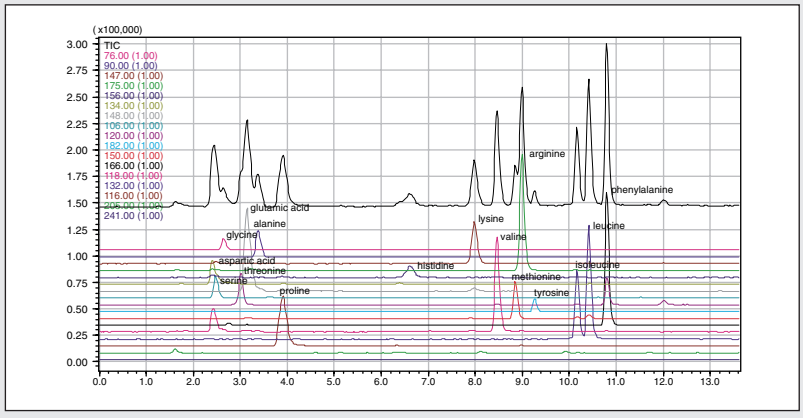


Fig. 2 SIM Chromatogram of Amino Acids in Soy Sauce

An LC system with a cation exchange column, pre- or post-column derivatization, and fluorescence detection is generally used for the analysis of amino acids. It offers compositional analysis of the protein content of culture fluid and protein structural analysis. However, a dedicated system is required and separation takes a comparatively long time. A more general-purpose, faster system is desirable.

The above shows an example of amino acid analysis by LC/MS. Electrospray ionization (ESI) is suitable for the analysis of amino acids, as amino acids are amphiprotic compounds that have a positive or negative charge according to the pH of the aqueous solution.

Fig. 1 shows the SIM chromatogram of the standard mixture of 18 amino acid components. The separation required for the quantitation of 18 amino acid components can be completed in approximately 12 minutes. Fig. 2 shows the analysis of the amino acids contained in soy sauce (diluted 250 times).

This type of system is widely used for the analysis of the free amino acids in vinegar or Japanese sake.

Data

Analysis of Compounds Related to Nucleic Acids

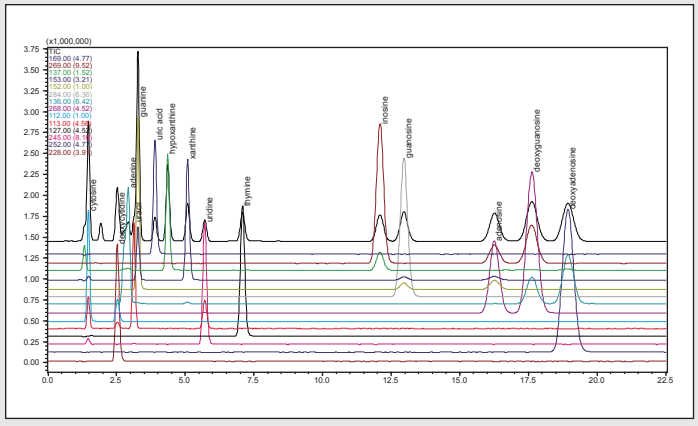


Fig. 2 SIM Chromatogram of Standard Mixture of Nucleic Acid Bases and Nucleotides

Nucleic acid bases and nucleotides are generally separated by ion-exchange or reverse-phase mode LC and detected by UV absorbance detection.
In the example introduced here, the mass information is acquired and the nucleic acid-related compound analyzed at high sensitivity by LC/MS.

Fig. 1 shows the structure and ESI mass spectrum of the purine base Adenine and of the nucleotide Adenosine.
Under acidic conditions in the positive ion mode, the protonated molecule $[M+H]^+$ is observed as a standard peak. Fig. 2 shows the LC/MS analysis results of a standard mixture of nucleic acids. SIM measurements were conducted using $[M+H]^+$ as the detected ion for each amino acid. Satisfactory separation of the 15 components was achieved.

Analytical Conditions

Column	: L-column ODS (150 mm L. × 2.1 mm I.D.)
Mobile phase A	: 0.1% acetic acid–water
Mobile phase B	: acetonitrile
Time program	: 1%B (0-20 min)–80%B (20.1-30 min)
Flow rate	: 0.2 mL/min
Injection volume	: 3 µL
Column temp.	: 30 °C
Probe voltage	: +4.5 kV (ESI-positive mode)
CDL temp.	: 200°C

Block heater temp.	: 200 °C
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 0.1 MPa
CDL voltage	: +15 V
Qarray DC voltage	: Scan mode
Qarray RF voltage	: Scan mode
SIM	: *m/z* 136, 137, 152, 153, 169, 228, 252, 268, 269, 284

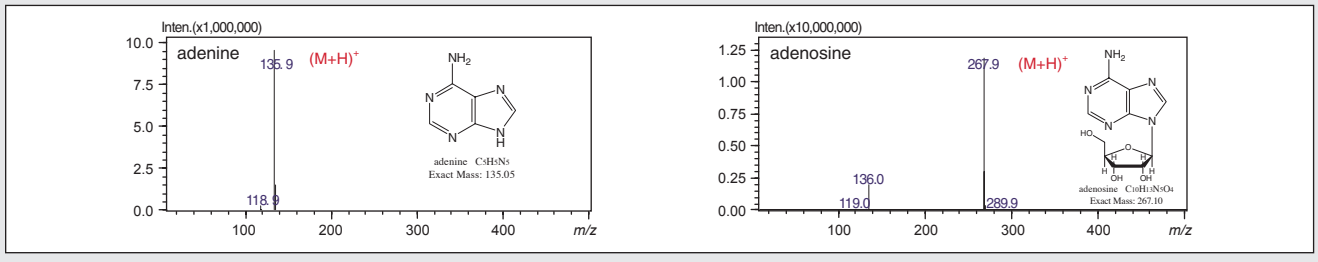


Fig. 1 Positive-Ion ESI Mass Spectrum of Adenine and Adenosine

Fig. 3 shows the analysis of purine bases and purine nucleotides in beer.
Beer was diluted 100 times in ultrapure water and filtered to create the analysis samples. Xanthine and Guanosine were detected in the beer (Fig. 3A), whereas they were virtually undetectable in the purine-cut, low-malt beer (Fig. 3B).

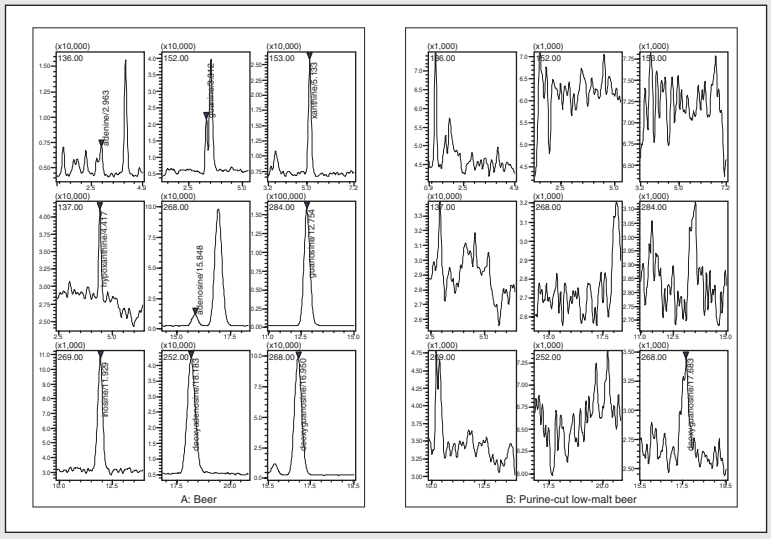


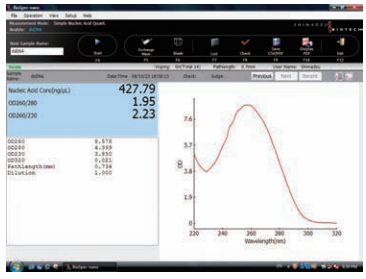
Fig. 3 SIM Chromatograms of Purine Bases and Nucleotides in Beer

Analysis of Proteins and Peptides

BioSpec-nano
Life Science Spectrophotometer

Resolving Dissatisfaction with the Simplified Quantitation of Nucleic Acids

BioSpec-nano is a specialized spectrophotometer for nucleic acid quantitation.
- To start the analysis, simply drop the sample on to the target and click a button.
- The instrument automatically performs measurements and wiping.
- It can analyze sample volumes of 1 to 2 µL.

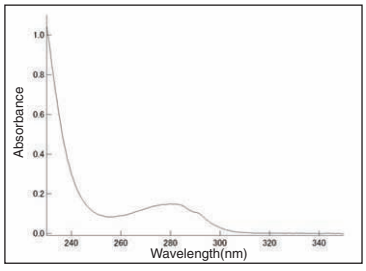


UV-1800
UV-VIS Spectrophotometer

Compact, High-Performance UV-VIS Spectrophotometer

- 1 nm resolution tops the general-purpose instrument class. The Czerny-Turner-mounted monochromator creates a compact but bright optical system.
- Data saved in the USB memory can be displayed and printed at a PC to allow standalone or PC controlled operation and improve the ease-of-use.
- Enhanced security by setting the user authority levels. Improved maintenance and servicing functions.
- Comprehensive validation functions. Automatic or semi-automatic investigation of the nine items prescribed in JIS K 0115 (wavelength accuracy, wavelength repeatability, photometric accuracy, photometric reproducibility, resolution, stray light, baseline stability, baseline flatness, and noise level).

Top-of-the-range models (UV-2550/2450, UV-3600) also available.



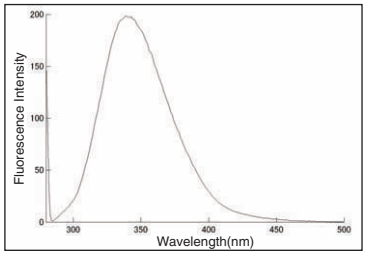
UV Analytical Conditions

Sample	: anti-apolipoprotein A-I mouse mAb (CALBIOCHEM)
Conc.	: 60 µg/mL
Slit width	: 1.0 nm
Scan range	: 220 to 350 nm
Scan speed	: medium
Sampling interval	: 0.5 nm

RF-5300PC
Spectrofluorophotometer

High-Sensitivity Spectrophotometer Supports Diverse Applications

- Digital signal processing and the bright optical system offered by the blazed holographic grating achieve an S/N ratio of at least 150 (bandwidth: 5 nm; Ex 350 nm; water Raman).
- High-speed scanning at up to 5,500 nm/min acquires the required spectrum in a few seconds.
- Configurable into an automatic system. Using the optional sipper unit permits the measurement of samples in multiple test tubes without transferring the sample from the test tube to the cell.
  Connecting the ASC-5 Autosampler permits automated measurement of up to 100 samples.
- Built-in instrument management functions. These include noise-level (S/N ratio) measurement functions and light source (Xenon lamp) operating time management functions. These functions always maintain the instrument in optimal status.



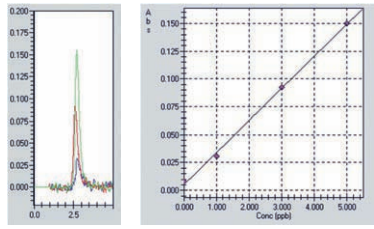
RF Analytical Conditions

Sample	: anti-apolipoprotein A-I mouse mAb (CALBIOCHEM)
Conc.	: 60 µg/mL
Spectrum mode	: emission
Excitation wavelength	: 280 nm
Scan range	: 280 to 500 nm
Bandwidth	: 3 nm (excitation/emission)
Scan speed	: slow
Sampling interval	: 1 nm
Sensitivity	: high

AA-7000 Series
Atomic Absorption Spectrophotometer

Shimadzu Atomic Absorption Spectrophotometers Accommodate Diverse Requirements

An atomic absorption spectrophotometer is used for research into the processing of water-soluble selenium in wastewater using microorganisms. It simplifies the quantitative analysis of the elements contained in a sample solution. The flame method is suitable for ppm-order measurements and the flameless method is suitable for the measurement of trace elements in the order of ppt to ppb.
The AA-7000 Series of atomic absorption spectrophotometers achieves ease-of-use, and offers a variety of powerful features, including enhanced high-sensitivity analysis, a system configuration with integrated flame/flameless methods, and the world's smallest installation footprint (dual-atomizer model).



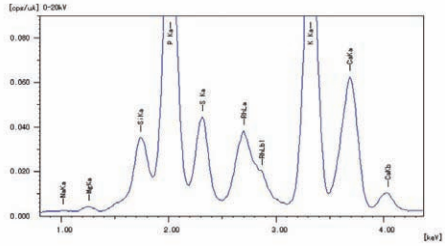
Measurement of Selenium (0 to 5 ppb)

EDX-GP
Energy Dispersive
X-Ray Fluorescence Spectrometer

μEDX Series
Energy Dispersive Micro X-Ray
Fluorescence Spectrometer

Simplified Elemental Analysis Without Pretreatment

The energy dispersive X-ray fluorescence spectrometer irradiates the sample with X-rays and measures the energy of the generated fluorescent X-rays to determine the type and content of the elements comprising the sample. EDX permits the non-destructive elemental analysis of samples in solid, powder, or liquid form and is used in a wide range of applications.



Qualitative Analysis of Brewer's Yeast Formulation

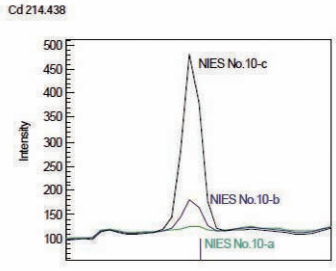
The μEDX Series significantly enhances the analysis sensitivity in minute areas that cannot be measured by conventional EDX. A newly developed X-ray focusing lens permits measurements of minute areas down to 50 μm diameter.

ICPE-9000
Multitype Inductively Coupled
Plasma Emission Spectrometer

ICPM-8500
Inductively Coupled Plasma
Mass Spectrometer

ICP Offers Simultaneous Multi-Element Analysis

The inductively coupled plasma emission spectrometer offers rapid qualitative and quantitative analysis of the element components contained in a sample solution. ICPE-9000 can perform batch elemental analysis at a range of concentrations, from trace-level harmful elements to large quantities of nutritional components.
The ICPEsolution Control and Data Processing Software can be used to allow anyone to obtain accurate analysis results.



Cadmium (Cd) Profile in Rice Flour-Unpolished
(NIES No. 10-a, 10-b, and 10-c) Standard Samples

The inductively coupled plasma mass spectrometer performs rapid qualitative and quantitative analysis of the ppt-level element components contained in a sample solution. It is an effective tool for identifying powders by measuring the isotope ratios.

JQA-0376

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