C10G-E089

SHIMADZU
Excellence in Science

Guide to Biopharmaceutical Solutions

—From Cell Line Optimization to Pharmacokinetics—

Solutions Designed for Biopharmaceutical Workflows

Cell Line Optimization

- Quantitation of Nucleic Acids — P. 4–7
- DNA/RNA Analysis — P. 8–9
- Colony Picking — P. 10–11

Culture

- Analysis of Metal Elements in Culture Solutions — P. 12–13
- Analysis of Chemical Components in Culture Solutions — P. 14–15
- Automating Pretreatment — P. 16–17

Purification

- Purification and Analysis — P. 18–19

Characterization

- Analysis of Primary and Protein Secondary Structures — P. 20–27
- Glycan Analysis — P. 28–33
- Protein Quantitation and Molecular Weight Determination — P. 34–37
- Aggregate Measurement — P. 38–41
- Stability Evaluation — P. 42–43

Quality Control

- Controlling Elemental Impurities — P. 44–45
- Blood Concentration Evaluation — P. 46–51
- Biomarker Discovery — P. 54–57

Pharmacokinetics

- Metabolite Analysis — P. 52–53

Quantitation of Nucleic Acids

Quantitation of Double-Stranded DNA

- Trace Measurement Using TrayCell and Nano Stick -

click here

Operating Principle and Features

The UV-1900i UV-VIS spectrophotometer features a space-saving and ergonomic hardware design. The user interface (UI) is displayed on a color touch panel to ensure the system status and operating procedures can be determined easily with a single glance. The Biomethod mode includes six types of built-in measurement conditions: 1. Nucleic acid quantitation, 2. Lowry method, 3. BCA method, 4. CBB (Bradford method), 5. Biuret method, and 6. UV method. These methods can be used to measure samples easily for given analytical objectives. The operation panel screenshot function can be used to easily extract measurement results without connecting to a computer. A 10 mm square cell requires a sample volume of approx. 4 mL, but the use of a TrayCell or Nano Stick cell enables measurement of micro sample quantities of approx. 2 to 4 µL.

Measurement Method

Double-Stranded DNA Measurement Method Using a TrayCell

Double-stranded DNA was prepared to create 27.5, 55, 110, 220, and 440 ng/µL standard samples (diluted with ultrapure water). Actual samples were prepared by ethanol precipitation of the same DNA. With the TrayCell, the optical path length can be changed to either 1.0 mm or 0.2 mm by switching between two types of caps. In this example, a cap with a 1.0 mm optical path length was used to measure 4 µL of dripped sample based on the conditions listed in Table 1 (Fig. 1).

Double-Stranded DNA Measurement Method Using a Nano Stick Accessory

Standard samples and actual samples of double-stranded DNA were prepared using the same method as described for the TrayCell above. The same measurement conditions were also used, as listed in Table 1. 3 µL sample volumes were measured with the 0.5 mm optical path length of the Nano Stick (Fig. 2).

Table 1 Measurement Conditions

Wavelength (Calibration curve):	260 nm, 320 nm
Wavelength range:	220 nm to 330 nm
Scan speed:	Low
Sampling pitch:	1.0 nm

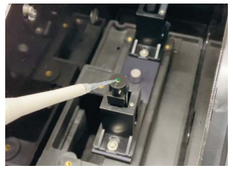


Fig. 1 TrayCell

1. Place the sample at the center of the silver area.

2. Install the cap.

3. Place the cuvette in the spectrophotometer.

4. Wipe off the sample.

Fig. 2 Using a Nano Stick Cell

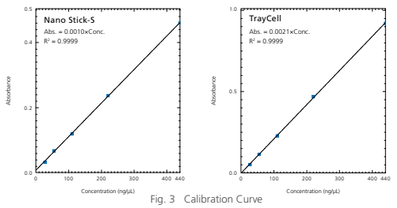


Fig. 3 Calibration Curve

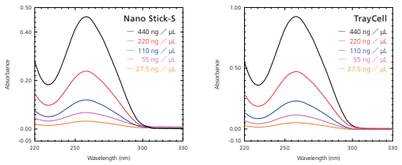


Fig. 4 Absorption Spectra of Lambda-DNA

Results

Calibration curves and UV spectral results from measurements using the TrayCell and Nano Stick are shown in Fig. 3 and Fig. 4. Both resulted in calibration curves with high linearity and good measurement accuracy, confirmed by correlation and CV values calculated from 10 repeated measurements of a 440 ng/µL sample.

Conclusion

TrayCell and Nano Stick accessories were used with a UV-1900i UV-VIS spectrophotometer to confirm that micro sample quantities on the order of several microliters can be measured accurately and easily.

Application Examples

- Evaluating DNA purity based on absorbance ratio
- Measuring DNA concentration
- Measuring protein concentration

index

UV-1900i

benefits

- Spectra can be acquired at ultra-fast scan speeds up to 29,000 nm/min.
- Sample volume of as low as 0.7 µL can be measured using a TrayCell or Nano Stick cell.
- Nucleic acid concentration can be easily determined using the built-in Biomethod mode.

Specifications

Instrument	UV-1900i
Sample volume	10 mm standard cell = 2.5 to 4.0 mL TrayCell = 0.7 to 10 µL, Nano Stick = 2 µL min.
Wavelength range	190 to 1,100 nm
Spectral bandwidth	1 nm
Light source	20 W halogen lamp and deuterium lamp Built-in light source auto position adjustment
Monochromator	LO-RAY-LIGH grade blazed holographic grating in Czerny-Turner mounting
Detector	Silicon photodiode
Sample compartment	Internal dimensions: W 110 × D 250 × H 115 mm
Distance between light beams	100 mm
Dimensions	W 450 × D 501 × H 244 mm
Weight	16.6 kg
Output device	USB memory (optional) Extended memory (optional) Data files saved in text format or UVPC format* *Files in UVPC format can be read with the UVProbe file viewer, which is a function of LabSolutions UV-Vis, or with UVProbe software.
Display	24-bit color touch screen Touch pen (standard included) Touch panel protective sheet (optional)

index

Quantitation of Nucleic Acids

Quantitation of Double-Stranded DNA Using BioSpec-nano

click here

Operating Principle and Features

The BioSpec-nano has two available optical path lengths, 0.2 mm and 0.7 mm, which enable quantitation of nucleic acids in very low sample volumes of 1 or 2 µL. Samples can also be measured using an optional cell with a 5 mm optical path length (for 2 mL volumes of dilute samples).
An automatic wiping function enables wiping the samples between measurements, eliminating the need to manually clean the sample stage and reducing cross contamination between samples.

Measurement Method

The sample consisted of purified dsDNA dissolved in Tris-EDTA (TE) buffer solution. The individual samples were prepared in the concentration ranges listed in Table 1 for each pathlength. Next, 10 successive measurements were conducted using each of the pathlengths and concentrations using the BioSpec-nano, and the OD (Optical Density, absorbance corresponding to the 10 mm pathlength) at 260 nm was determined. The Y-axis values (Measured OD260) in Fig. 1, 2, and 3 correspond to BioSpec-nano measurement values. The standard value (Corrected OD260, X-axis in each figure) for determining the accuracy was obtained using the Shimadzu Ultraviolet-Visible spectrophotometer, an appropriately diluted sample and a 1 mm pathlength cell. The linearities of Fig. 1, 2 and 3 indicate the linearity of the standard values, and the deviation from each of the straight lines correspond to OD error.

Results

Analysis Results with 0.2 mm Pathlength

The correlation coefficient of 0.999 for OD260 was obtained with respect to the standard value (Fig. 1). When the OD value was greater than 5 (250 ng/µL dsDNA), the measurement repeatability as CV (%) was less than 1.4 %, and the OD error (%) was from -5.4 % to 2.8 %. The data are shown in Fig. 1.

Analysis Results with 0.7 mm Pathlength

The correlation coefficient of 0.999 for OD260 was obtained with respect to the standard value (Fig. 2). When the OD value was greater than 1.4 (70 ng/µL dsDNA), the measurement repeatability as CV (%) was less than 1.4 %, and the OD error (%) was from -8.6 % to 4.4 %. The data are shown in Fig. 2.

Analysis Results with 5 mm Pathlength Cell

The correlation coefficient of 0.999 for OD260 was obtained with respect to the standard value (Fig. 3). When the OD value was greater than 0.2 (70 ng/µL dsDNA), the measurement repeatability as CV (%) was less than 0.6 %, and the OD error (%) was from -1.6 % to 3.6 %. The data are shown in Fig. 3.

Performance of Automatic Wiping in Nucleic Acid Quantitation

We alternated measurement of purified dsDNA (11.7 OD, 578 ng/µL) and TE buffer solution using a 0.7 mm pathlength, 3 µL sample volume, and 1 wipe operation between measurements. Carryover (%) of dsDNA to the TE buffer solution was used as an index of the automatic wiping performance.

Carryover (%)

$$= 100\times \frac{[(\text{Nucleic acid concentration in TE measurement})]}{[(\text{Nucleic acid concentration in dsDNA measurement})]} \cdots (1)$$

Given the steps involved in one set, including measuring double-stranded DNA → wiping → adding TE buffer → wiping, repeating that set 60 times resulted in carryover (%) that remained 0.3 % or less, which confirmed that sample carryover in the sample area when using automatic wiping is extremely low.

Table 1 Analytical Conditions

Pathlength 0.2 mm	Sample concentration	50 to 3700 ng/µL
	Sample volume	1 µL
Pathlength 0.7 mm	Sample concentration	15 to 1000 ng/µL
	Sample volume	2 µL
5 mm Pathlength Cell (option)	Sample concentration	2 to 150 ng/µL
	Sample volume	2 mL

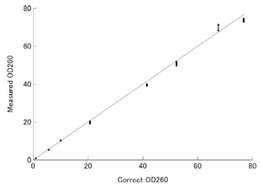


Fig. 1 Analysis Results with 0.2 mm Pathlength

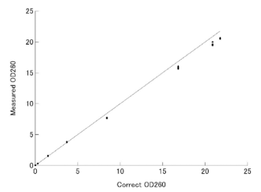


Fig. 2 Analysis Results with 0.7 mm Pathlength

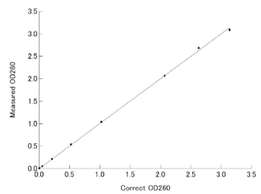


Fig. 3 Analysis Results with Optional 5 mm Pathlength Cell

Summary

BioSpec-nano is capable of simple and excellent measurement linearity, reproducibility, and accuracy with a sample volume of 1 to 2 µL for optical pathlengths of 0.2 mm and 0.7 mm, respectively.

Application Examples

- Measuring single-strand DNA concentration
- Measuring RNA concentrations
- Measuring protein concentration (refer to p. 34)

BioSpec-nano

benefits

- Measure the concentration or check the purity of double-stranded DNA extracts.
- Measure sample quantities as small as 1 µL.
- Automatic wiping function enables a low-carryover system.

With the automatic wiping function, never forget to wipe off samples.

Specifications

Instrument	BioSpec-nano
Wavelength range	220 to 800 nm
Spectrum bandwidth	3 nm
Wavelength accuracy	±1 nm
Pathlength	0.2 mm, 0.7 mm
Photometric value unit	OD (Optical Density), absorbance converted with 10 mm pathlength
Sample volume	1 µL min. (pathlength: 0.2 mm) 2 µL min. (pathlength: 0.7 mm)
Light source	Xenon flash lamp
Monochromator	Holographic grating
Detector	Photo diode array
Auto wiping function	Provided
Spectrum measuring time	3 sec
Quantitation range	Pathlength 0.2 mm, 1 to 75 OD, 50 to 3,700 ng/µL Pathlength 0.7 mm, 0.3 to 21 OD, 15 to 1,000 ng/µL Optional 5 mm pathlength cell, 0.04 to 3 OD
Dimensions	W 210 mm × D 214 mm × H 417 mm
Weight	7 kg
Analysis mode	Simple nucleic acid quantitation, labeled nucleic acid quantitation, protein quantitation, labeled protein quantitation, photometric measurement

Note: The droplet formation status will affect analysis results. Measure quantities that are large enough to enable proper droplet formation.

Electrophoresis for DNA/RNA Analysis

Checking for Genome Editing Mutations by Heteroduplex Mobility Assay

click here

Operating Principle and Features

MultiNA is an automatic electrophoresis system that uses a microchip to measure the size of DNA or RNA. It automates all steps, such as creating the gel for agarose gel electrophoresis, applying the sample, electrophoresing, staining, detecting, and rinsing. MultiNA uses dedicated reagents, fluorescent dyes, and microchips to fully automate analysis and achieve quick, easy, and high-sensitivity electrophoresis (Fig. 1).

Application

When Transcription Activator-Like Effector Nuclease (TALEN) or a CRISPR/Cas system is used to break a genome at any particular point in a sequence, the cell will repair the double-stranded DNA break.
Genome editing is a technology that uses the repair errors that occur during repairing to modify genomes by inserting or deleting code in the original sequence.
One technique used to verify whether the genome editing process successfully introduced the intended genetic modification is the heteroduplex mobility assay (HMA). It uses electrophoresis mobility to discriminate between homoduplex and heteroduplex DNA, which have different steric structures.

Measurement Method and Result

After the mutation has been induced in an individual, PCR is conducted for the area in the vicinity of the deletion/insertion. The PCR product is denatured, then reannealed to form a heteroduplex product. Then, by checking the migration pattern of the sample using the MultiNA, the presence of short deletions can be verified by means of the structural change, which would be difficult to determine solely by comparing differences in chain length (Fig. 2).

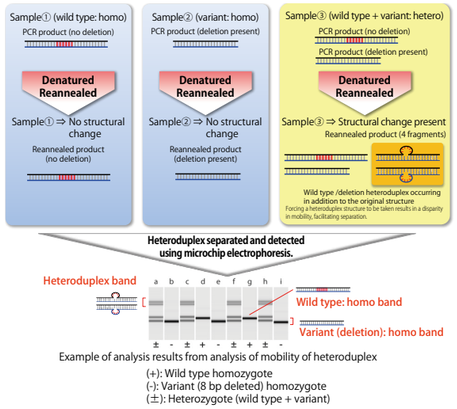


Fig. 2 HMA Principles and Analysis Procedures

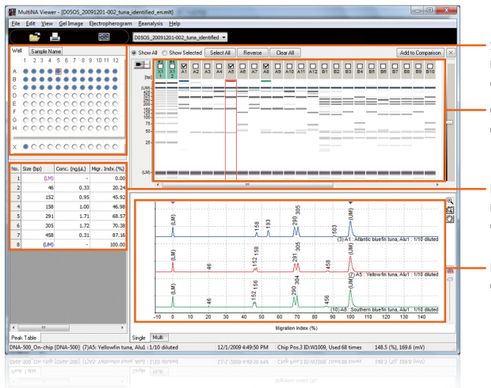


Fig. 1 Displaying Analysis Results in the MultiNA Viewer

Conclusion

The MultiNA automatic analysis platform solves previous shortcomings of agarose electrophoresis. It provides an easy way to check the presence and size of DNA/RNA with good reproducibility.

Application Examples

- Verify mutations created by genome editing
- Check libraries of next-generation sequencers
- Genotyping or detecting microorganisms or viruses

This article was prepared with help from Assistant Professor Masato Kinoshita of the Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University.

index

MCE-202 MultiNA

benefits

- Reduces the cost and time involved in analysis
- Enables fully automatic batch analysis of up to 108 samples
- Achieves high sensitivity, high resolution, and high reproducibility

Specifications

Instrument	MCE-202 MultiNA
Sample rack	Compatible with 96-well PCR plate (An aluminum sheet can be applied to prevent sample evaporation.) and 12/8-strip PCR tube (Shimadzu recommended product)
Microchip	Quartz, 23 mm separation channel length, on-chip electrodes (insert up to four microchips)
Pretreatment	Automatic sample injection, automatic separation buffer replenishing, automatic chip cleaning
Electrophoresis voltage	Max. rated voltage: 1.5 kV, max. current: 250 µA
Detection method	LED-excited fluorescence detector (470 nm excitation wavelength) <Class 1 LED product>
Loaded samples	Up to 108 samples
Separation size range (reagent kits dedicated for MultiNA)	25 to 500 bp (DNA-500 Kit) 100 to 1000 bp (DNA-1000 Kit) 100 to 2500 bp (DNA-2500 Kit) 100 to 12000 bp (DNA-12000 Kit) Up to 28S rRNA (5.0 knt) (RNA Kit)
Microchip rinsing	Chip rinsing kit RA
Sample volume	5 µL
Quantitation range	DNA analysis: 0.5 to 50 ng/µL (at 10 mM Tris-HCl, containing 50 mM KCl and 1.5 mM $MgCl_2$) RNA analysis: 25 to 500 ng/µL (total RNA), 25 to 250 ng/µL (mRNA) (10 mM Tris-HCl buffer, containing 1 mM EDTA)
External dimensions	W 415 mm × D 545 mm × H 508 mm
Weight	43 kg
Power supply	100 to 120 V, 220 to 240 (CE Marking) 300 VA max.
Controller	Creating analysis schedules, real-time control, automatic analysis pretreatment, automatic analysis post-treatment, automatic error processing, analysis log management, analysis performance checks
Data processing	Batch display/detailed display of gel images/pherograms, automatic quantitation and size prediction by size markers, data searching, data import/export, manual editing and re-analysis Changes in average size and concentration with respect to smear samples (during smear analysis)
Reports	Multilevel data display, tree display of samples/files, RNA structural comparison, analysis performance check results, analysis log

Note: MCE-202 MutiNA is currently not available in US, EU and UK.

index

Cell Colony Picking

Cell Colony Picking Method Used to Automate Picking Operations for Cell Genome Editing

click here

Operating Principle and Features

CELL PICKER has a technology to aspirate and discharge liquids. After visually deciding the target cell colony, a button is pressed to automatically attach a pipette tip to the end of the nozzle and reliably move the tip close to the target cell colony. When the tip scrapes off the cell colony, the measuring pump simultaneously activates to aspirate and then discharge the cell colony and a small amount of the medium. Using a tablet computer for observations and operations can reduce the amount of work involved in operations.

Procedure and Cultivation Parameters

A 6-well plate was seeded with 1×10^4 to 1×10^6 cells/well of human colon cancer cells (HCT116 adherent cell line). After cultivating the cells for six days, the CELL PICKER was used to pick cells and seed a 96-well plate. Then the same cultivation parameters were used to cultivate the seeded cells for six days, after which the cell adhesion and proliferation were checked. The operation process flow is illustrated in Fig. 1.

Table 1 Adherent Cell Cultivation Parameters

Cells:	HCT116 cell line (human colon cancer cells)
Culture medium:	McCoy's 5A medium with 10 % FBS, 2 mM glutamine added
Coating:	Gelatin solution
Cultivation parameters:	Let stand at 37 °C and 5 % CO_2

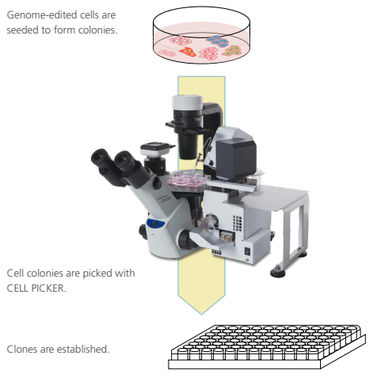


Fig. 1 Workflow image

Results

Cell colonies before and after picking are shown in Fig. 2. The picked cells after cell proliferation are shown in Fig. 3.
100 % of the seeded wells produced adherent cell cultures.
This example confirmed that cells can be picked and seeded without causing cell damage.

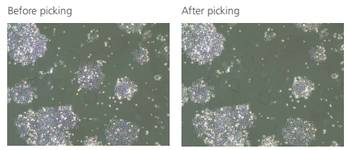
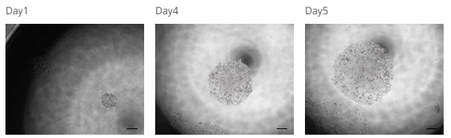


Fig. 2 Cell colonies before and after picking

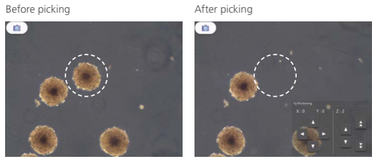
Application Examples

- **Picking for establishing iPS cells**
  This confirmed that iPS cell colonies can be cultivated continuously while maintaining their undifferentiated state.
- **Collecting floating cell clusters (Spheroids)**
  The system can also be used for the purpose of collecting spheroids created by 3D cell culturing.
  It can pick a single spheroid from among multiple spheroids in an HEK293 cell line (400 to 500 µm) (Fig. 4).



Note: These colonies are different than shown in "Cell colonies before and after picking."
Scale bar: 200 µm

Fig. 3 Cell proliferation after picking



The white dashed circles in the images are not shown in the software.
Note: The indicated operations require special operating software.

Fig. 4 Before and after picking spheroids

CELL PICKER

benefits

- Automation of manual steps enables reliable picking operations.
- Operations can be recorded easily.
- The compact space-saving design is ideal for installation in cramped laboratories.

Specifications

Instrument	CELL PICKER
Microscope (recommended)	Olympus CKX53
Pipette tip volume	200 µL
Recommended pipette tip	QSP (Thermo Fisher Scientific) TW110-96RNS-Q
Cell culture vessels	6 well plate: FALCON_353046 / IWAKI_3810-006 6 cm dish: FALCON_353002 / IWAKI_3010-060 10 cm dish: FALCON_353003 / IWAKI_3020-100
Suction amount	Picking mode: 5/10/15 µL Removal mode: 5 µL
Dimensions	W 280 mm × D 350 mm × H 400 mm (not included microscope)
Weight	Approx. 8.5 kg
Power supply	100 to 240 V AC, Frequency: 50/60 Hz, Power consumption: 75 VA
Operating environment	Temperature: 10 to 35 °C Humidity: 20 to 85 % RH

Note: A table PC for operation is required separately.

Metal Elements Easily Quantified during Culturing

Monitoring of Metal Elements in Cell Culture Supernatant using Atomic Absorption Spectrometry

click here

Operating Principle and Features

Atomic absorption spectrometry involves atomizing elements at high temperature to quantitate element concentrations based on the absorption of specific light wavelengths during atomization.
There are two main atomization methods: (1) the electric thermal method, which involves generating heat with an electrical current (high sensitivity), or (2) the flame method, which involves heating with a flammable gas flame. (Table 1 shows a comparison.) Either method can be used in AA-7000 systems, which include an auto-atomizer changer (AAC) that can be used to automatically switch between the methods for measurements.

Measurement Method and Conditions

The high concentrations of Mg and Zn were measured using the flame method and trace elements (Cu, Mn, Co, and Fe) using the electric thermal method, based on the analytical conditions indicated in Tables 2 and 3.
CHO cells were inoculated in a 125 mL flask and cultivated by shaking for four days. Every 24 hours, from immediately after starting cultivation, 1 mL of the cell culture fluid was sampled, removed cells by centrifugation, and then the supernatant was collected. Samples were diluted by 20 times for Cu, Mn, and Zn, 40 times for Co and Fe, and 500 times for Mg before analysis (nitric acid was diluted to 0.5 v/v%). Standard solutions for each element were prepared by diluting the standard solution for atomic absorption spectrometry (1000 mg/L). The nitric acid concentration was prepared to 0.5 v/v%. The calibration curve method was used for all analyses.

Results

The calibration curve coefficient of correlation was r = 0.999 or higher for all components. A spike-and-recovery test was performed for each element by adding a standard solution with a fixed concentration. (The additive recovery rate equals the concentration difference between spiked and unspiked samples divided by the additive concentration.) Test results were roughly within 100 ±10 %, which is an excellent additive recovery rate.
The electric thermal method and flame method were also used to monitor time-series changes in culture supernatant concentrations for each sample. Resulting peak profiles and time-series concentration changes in the culture supernatant obtained by the two methods are shown in Figs. 1 and 2.

Conclusion

The concentrations of metal elements in a cell culture supernatant were measured using an AA-7000 atomic absorption spectrophotometer, which can measure samples using two types of atomic absorption spectrometry methods, electric thermal and flame.
Time-course changes in metal element concentrations can be monitored using only a simple pretreatment step of diluting the cell culture supernatant.

Application Example (Shimadzu Application News No.)

- Analysis of metallic elements in cell culture medium (A634)

Table 1 Comparison of Atomization Methods

	Electric thermal method	Flame method
Sensitivity	ppt to ppb	ppb to ppm
Atomization efficiency	90 % or more	Approx. 10 %
Required sample/analysis	5 to 50 µL	1 to 2 mL
Analysis time/analysis	2 to 5 min	5 to 10 sec
Repeatability	RSD 3 % (approx.)	RSD 1 % (approx.)

Table 2 Analytical conditions of the electric thermal method

	Analysis wavelength (nm)	Slit width (nm)	Ashing temp.	Atomization temp.	Lighting mode	Tube type
Cu	324.8	0.7	800 °C	2500 °C	BGC-D2	Platform tube
Mn	279.5	0.2		2200 °C		
Co	240.7			2300 °C		
Fe	248.3			2300 °C		

Table 3 Analysis conditions of the flame method

	Analysis wavelength (nm)	Slit width (nm)	Lighting mode	Flame type	C_2H_2 flowrate
Zn	213.9	0.7	BGC-D2	Air – Acetylene	2.0 L/min
Mg	285.2				1.8 L/min

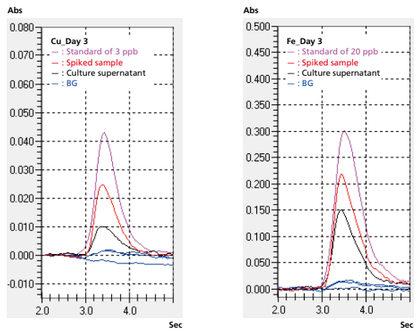
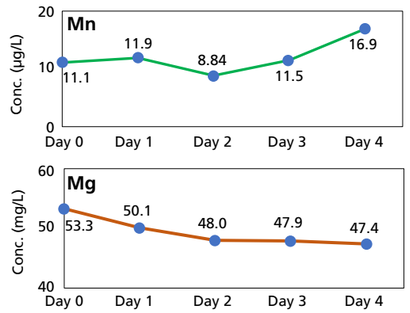


Fig. 1 Peak profile



Note: Value obtained by converting the measurement value to one corresponding to stock solution of cell culture supernatant.

Fig. 2 Time course of Mn and Mg concentration in culture supernatant

index

AA-7000 Series

benefits

- Metal elements in culture media can be analyzed without any complicated pretreatment steps.
- Multiple trace metal elements can be quantified inexpensively and easily.
- The system supports the electric thermal method, flame method, or automatically switching between the methods.

Specifications

	Instrument	AA-7000F/AAC	
	Wavelength range	185.0 to 900.0 nm	
	Bandwidth	0.2, 0.7, 1.3, 2.0 L nm (4-step automatic switching)	
	Background correction method	BGC-SR (high-speed self-reversal method) (185.0 to 900.0 nm), BGC-D2 (D2 lamp method) (185.0 to 430.0 nm)	
	Lamp mode	EMISSION, NON-BGC, BGC-D2, BGC-SR	
	Measurement mode	Flame continuous method, flame micro sampling method, furnace method, flame emission method	
	Maximum reagent / sample positions	Reagents: 8 positions, Samples: 60 positions (when using an autosampler)	
	Digital recording	Management by login ID and password, control user access authority by user level, log record, audit trail, electronic signatures	
	Positioning	Automatic flame/furnace switching by motor	
	Dimensions and weight	W 700 × D 588 × H 714 mm, 76 kg (Autosampler is not included.)	
Flame	Burner head	Titanium 10 cm slot (5 cm titanium slot for N_2O–C_2H_2 flame available as an option)	
	Nebulizer	Pt-Ir capillary, PTFE orifice, ceramic impact bead (capable of handling hydrofluoric acid)	
	Type	Air–C_2H_2, N_2O–C_2H_2	
	Safety measures	Automatic gas leak check, automatic Air–N_2O switching as C_2H_2 flowrate increases, flame monitor, prevention of wrong burner head use, gas pressure monitor, drain tank level monitor, automatic flame extinction upon power outage or sudden power interruption, automatic flame extinction via flame vibration sensor, internal fan stop sensor	
Furnace	Heating control system	Drying: Digital current control with automatic temperature calibration function Ashing, Atomization: Digital temperature control via optical sensor	
	Carryover	Rinse port: Less than 0.0001	Mixing port: Less than 0.00001
	Auto dilution / re-analysis	For measurement result on unknown samples · If extrapolation of calibration curve is possible: automatic calculation of dilution rate and dilution to bring concentration within calibration curve range · If extrapolation of calibration curve is not possible: dilution rate fixed at 10×	
	Safety measures	Cooling water flowrate monitor, gas pressure monitor, overcurrent protection unit (double check by circuit protector and optical sensor), furnace block cooling check	

index

Monitoring Components in Cell Culture Solutions

Simultaneous Analysis of Components in CHO Cell Culture Supernatant for Optimization of the Culture Process

click here

Operating Principle and Features

Triple Quad LCMS-8060 systems feature UF technology that enables both high sensitivity and high speed. Due to high-speed scanning and high-speed positive-negative ionization switching capability, the system can simultaneously analyze multiple metabolites with a wide variety of chemical properties. The cell culture profiling method package is an analysis method optimized for analyzing multiple components in culture supernatant solutions. It enables analyzing up to 125 compounds (refer to product specifications) in 20 minutes or less (Fig. 1).
This example describes monitoring the components in CHO cell culture supernatant over time.

Measurement Method and Results

CHO cell culture solutions were sampled every 24 hours and supernatants obtained by centrifugation. The supernatant was deproteinized and diluted with ultrapure water before analysis.
Analytical results over a time course are graphically represented in Fig. 2.
Important cell culture factors such as consumption of nutrients during cell proliferation and metabolic secretion can be monitored.

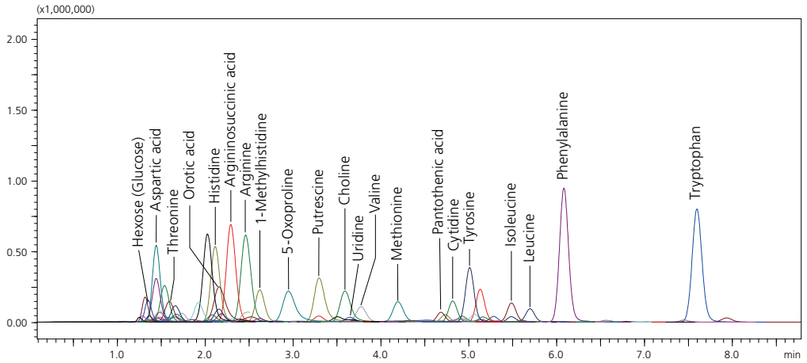


Fig. 1 MRM Chromatogram

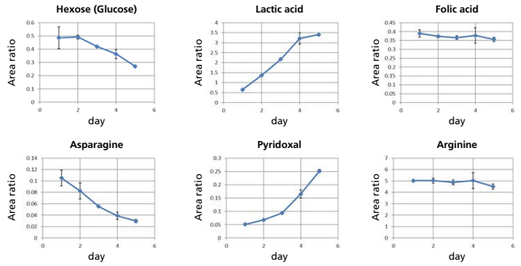


Fig. 2 Time-Series Changes in Each Component

Conclusion

Rapid and comprehensive cell culture media analysis for the determination of nutrients and metabolites is possible.

Application Examples

- Spent media analysis
- Qualitative and quantitative analysis of culture media, bovine serum, and other samples (requires calibration curve preparation)
- Metabolomic analysis of culture supernatant and body fluids

Nexera X3 UHPLC + LCMS-8060 + Cell Culture Profiling

benefits

- Simultaneous analysis of up to 125 amino acids, vitamins, nucleic acids, or other compounds contained in the culture supernatant within 20 minutes.
- Sensitivity levels have been specified based on the concentration of target components being measured, which can reduce the work involved in creating a series of dilutions.
- Time-series monitoring of multiple components provides powerful support for optimizing cultivation parameters.

Specifications

Software	LC/MS/MS Method Package for Cell Culture Profiling Ver.2
LC unit	Nexera X3 (SCL-40, LC-40BX3, CTO-40S, SIL-40C X3, MR20 µL mixer)
MS unit	LCMS-8045/-8050/-8060
Analysis cycle	Less than 20 minutes per sample
Registered compounds	125 compounds + internal standard substance (2-isopropylmalic acid) Amino acids and metabolites 60 compounds Nucleic acids and metabolites 31 compounds Vitamins 15 compounds Sugars 4 compounds Other (organic acids, etc.) 15 compounds
Separation mode	Reversed
Detection mode	MRM (positive/negative)

Cell Culture Media Analysis Platform

Automates Processes from Pretreatment to LC/MS/MS Measurement for Culture Supernatant Analysis

click here

Operating Principle and Features

The C2MAP-2030 is an automatic pretreatment system for removing proteins from culture supernatants by suction filtering the proteins precipitated by adding an organic solvent. Deproteinized culture supernatant samples are automatically transferred to the HPLC autosampler, where they are dispensed onto a microtiter plate (MTP) for storage. Those samples are automatically supplied for LC/MS/MS measurement, where 95 components are analyzed simultaneously using the Cell Culture Profiling Method (equivalent to Ver. 1). After peak integration, time-series data for each component can be visualized easily (Fig. 1) by loading the data file into the C2MAP TRENDS software included with the C2MAP-2030 system.
By connecting the C2MAP automatic pretreatment system to the LC-MS/MS system, samples can be analyzed seamlessly. Because sample information is linked to measurement data files, the C2MAP system can also reduce human error, such as from loading the wrong sample. Manual methods can cause variations in data quality, but automated equipment helps ensure any operator can acquire data with good repeatability (Fig. 2). Automation can also reduce operational hours (Fig. 3).

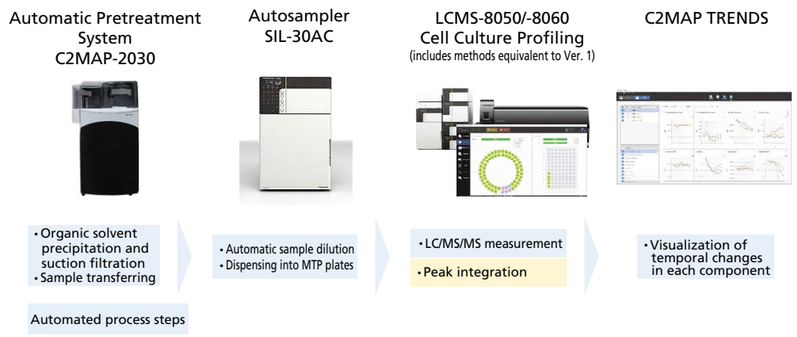


Fig. 1 Analysis Process Flow with C2MAP System

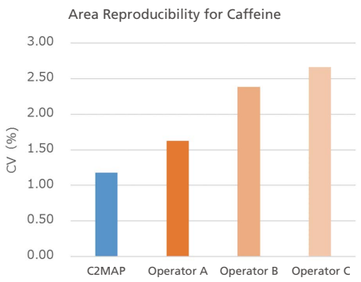


Fig. 2 Comparison of Analysis Repeatability

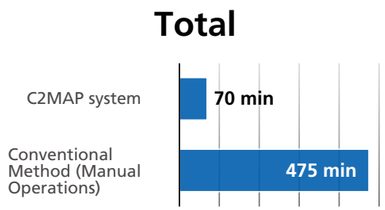


Fig. 3 Comparison of Operator Labor Hours

Conclusion

The C2MAP system can automate the deproteinization process for up to 65 culture supernatant samples.
By linking it to an LC-MS system, the entire process from pretreatment to LC/MS/MS measurement can be executed seamlessly.

C2MAP Cell Culture Media Analysis Platform System

benefits

- Automating culture supernatant analysis processes from pretreatment to LC/MS measurement ensures anyone can acquire high-quality data.
- Dedicated control software makes it easy to link samples to measurement results.
- The system can be operated using only the modules necessary for automatic pretreatment, while the LC-MS/MS system is also used for a separate analysis.

Specifications

Instrument	C2MAP-2030
LC unit	Nexera X2 (CBM-20A, LC-30AD, CTO-20AC, SIL-30AC, MR 20 µL mixer, and other units)
MS unit	LCMS-8050/-8060
Required sample quantities	400 to 500 µL (or 100 to 250 µL using optional rack)
Processing time	10.8 min per sample (17 min per sample for LC/MS measurements)
Pretreatment processes	Add internal standard, sample, and organic solvent, mix, filter by suction filtration, and transfer sample after processing
Pretreatment methods	Batch mode: Starts LC/MS measurement after pretreatment is finished for all samples. Sequential mode: Each sample processed successively in parallel. Pretreatment mode: All samples are pretreated only.
Max. number of samples	65 (or 64 using the sequential mode)
External dimensions	W 670 × D 700 × H 1,190 mm (C2MAP-2030 only)
Weight	185 kg (C2MAP-2030 only)

Automating Steps from Preparative Purification to Product Evaluation

Seamless Analysis from Purification of IgG in Human Blood Plasma to SEC Evaluation

Operating Principle and Features

Sodium chloride and other halogen ions essential for biopharmaceutical analysis are highly corrosive to metals. To eliminate this concern, the Prominence inert LC system uses PEEK or other polymer materials for all parts in contact with liquids, thereby ensuring worry-free operation.
The liquid handler (LH-40) is an integrated autosampler and fraction collector. That means samples acquired during the first analysis can be injected directly into the second analysis without having to transfer them from a fraction collector. For example, with this system, the target protein is purified by an affinity column and fractionated at the first step, and the fractionated protein is re-injected for SEC analysis at the second step. These two steps can be done by just specifying the method and fraction.

Measurement Method and Conditions

5 mL of commercial human plasma was diluted 5-fold with mobile phase in a 15 mL tube and the tube was placed in the LH-40 rack. This sample was purified by affinity chromatography with an IgG purification column following the conditions in Table 1. The elution fractions were collected on the 96-deep-well plate set in the liquid handler. Then, 100 µL of the fraction involving the peak's top point was analyzed by size exclusion chromatography (SEC) following the conditions in Table 2.

Table 1 Analytical Conditions of Affinity Purification

Column:	HiTrap rProtein A FF (1 mL) (made by Cytiva)
Mobile phase A:	10 mmol/L (sodium) phosphate buffer pH 6.9
Mobile phase B:	100 mmol/L (sodium) citrate buffer pH 4.0
Time Program (B. Conc.):	0% (0 – 10 min) → →100% (10.01 - 20 min) → →0% (20.01 – 35 min)
Flowrate:	1.0 mL/min
Column Temp.:	15 °C
Injection Volume:	5 mL
Detection:	SPD-20A (280 nm)
Flow Cell:	Inert flow cell

Table 2 Analytical Conditions of SEC analysis

Column:	Shim-pack Bio Diol-300 (300 mm × 4.6 mm I.D., 5 µm)
Guard Column:	Shim-Pack Bio Diol-300 (G) (30 mm × 8.0 mm I.D., 5 µm)
Mobile phase A:	10 mmol/L (sodium) phosphate buffer pH 6.9
Flowrate:	0.5 mL/min
Column Temp.:	15 °C
Injection Volume:	100 µL
Detection:	SPD-20A (280 nm)
Flow Cell:	Inert flow cell

Results

The IgG peak obtained from affinity purification in step 1 and the peak obtained by SEC analysis in step 2 (Fig. 1) were evaluated by performing SDS-PAGE. That resulted in detecting H and L-chain bands for the target IgG (Fig. 2).

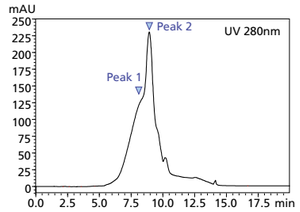


Fig. 1 Chromatogram of SEC analysis

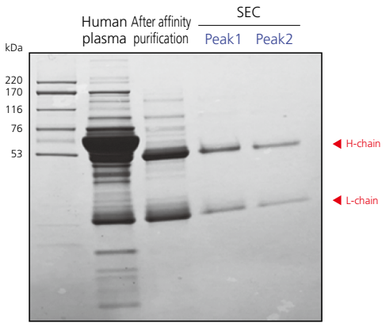


Fig. 2 SDS-PAGE (Reducing) Results

Conclusion

By simply setting a sample to the liquid handler (LH-40) installed in the LC system, the system can not only purify the sample, but also seamlessly further analyze fractions from the sample. For routine work with a prespecified target, it is possible to analyze only the target fraction. By adding a column switching valve and increasing the number of columns, the system can also be used to screen purification parameters or purify samples in multiple steps. After fractions are collected in a 96-well plate, they can be used directly for SDS-PAGE, ELISA, or various other analytical methods.

Application Examples

- Discovering and checking the quality of proteins in cell cultures
- Optimizing cultivation parameters
- Evaluating proteins in blood

Prominence Inert LC System + LH-40 Liquid Handler

benefits

- By selecting the target peak after fractionation, fractions can be automatically reinjected for analysis.
- A column switching valve allows automatic switching between columns for purification or analysis.
- Useful for optimizing cultivation parameters or other scenarios that involve comparing large numbers of samples.

Compatible with 96-well plates and a wide variety of test tubes and other containers

Specifications

Prominence inert LC system	System controller	CBM-20A	
	Solvent delivery unit	Two LC-20Ai units	
	Degassing unit	DGU-20A5R	
	Column oven	CTO-20AC	
	Mixer	PEEK mixer	228-45093-92
	UV-VIS detector	SPD-20A	
		Flow cell for inert LC system	228-33338091
	High-pressure flow channel switching valve	FCV-12AHi	
	VP option box		228-65512-58
LH-40 liquid handler	LH-40 liquid handler, main unit		228-65506-41
	LH valve kit, preparative		228-75605-42
	Syringe kit, 20 mL		228-64173-44
	Sample coil, 5 mL		228-39389-94
	Analysis kit		228-75587-41
	Rinse pump		228-75586-41
	Sample rack		228-75268-41
	Rack kit, D16		228-75604-49
	Rack kit, MTP		228-75604-40
Examples of SEC analytical column	Shim-Pack Bio Diol-300	(300 mm × 4.6 mm I.D., 5 µm)	227-31010-04
	Shim-Pack Bio Diol-300 (G)	(30 mm × 8.0 mm I.D., 5 µm)	227-31010-06

Protein Primary Structure Analysis

N-Terminal Amino Acid Sequencing of Mouse IgG Using PPSQ-51A/53A Gradient System

click here

Operating Principle and Features

The PPSQ protein sequencer automates the Edman degradation process.
Although using Edman degradation to determine amino acid sequences is very time-consuming, the reliability of the resulting amino acid sequences is very high, making it especially useful for protein amino acid sequencing when no database has been built. PTH-amino acids obtained by Edman degradation are analyzed by isocratic or gradient elution.

Measurement Method and Conditions

To operate the sequencer, proteins or peptides to be analyzed are applied to a glass filter treated with polybrene or transferred to a PVDF membrane. After electrophoresis, they are stained and placed in the reactor with an excised protein spot. After that, they can be analyzed automatically. In this example, samples were prepared by reducing 2 pmol of IgG from mouse serum, separating that into H and L-chains by SDS-PAGE, transferring the chains onto a PVDF membrane, staining, destaining, and then excising the resulting bands (Fig. 1 and 2). The IgG is reduced and separated into H and L-chains. The H and L-chains were separated and purified based on conditions indicated in Table 1 and then their amino acid sequences were analyzed (Fig. 3).

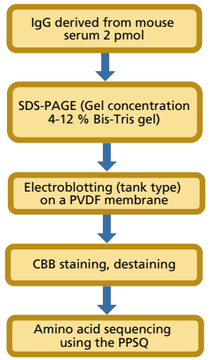


Fig. 1 Protocol for Analysis

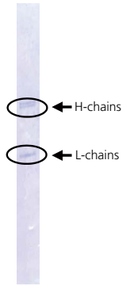


Fig. 2 PVDF Membrane after Electroblotting

Table 1 Analysis Conditions (Gradient System)

Column:	Wakopak Wakosil PTH-GR (S-PSQ, 250 mm×2.0 mm I.D.)
Mobile phase A:	PTH-amino Acids Mobile Phase A (for Gradient Elution)
Mobile phase B:	PTH-amino Acids Mobile Phase B (for Gradient Elution)
Flowrate:	0.3 mL/min
Column Temp.:	35 °C
Detection:	SPD-M30A (269 nm)
Flow Cell:	High Sensitivity Flow cell

Results

The amino acids in L-chains can be identified to 13 residues from the N-terminal, as Asp-Ile-Gln-Met-Thr-Gln-Ser-Pro-Ala-Ser-Leu-Ser-Ala(Val). A database search confirms that the sequence is for an immunoglobulin kappa light chain (Fig. 2).

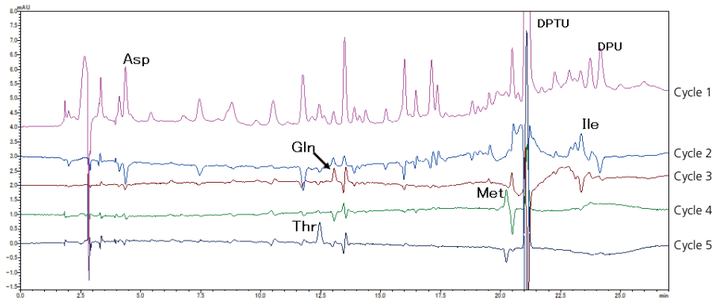


Fig. 3 Chromatogram of L-Chain (Raw Chromatogram from First Cycle and Difference Chromatograms from Cycles 2 to 5)

Conclusion

PPSQ-51A/53A systems can determine N-terminal sequences easily and accurately. The gradient system can detect peaks with approx. 3 to 5 times higher overall peak height than when using isocratic elution, which means amino acid sequences can be determined for even trace quantities of protein.

Application Examples

- Identifying the primary sequence of peptides
- Identifying the presence and position of S-S bonds
- Identifying post-translational modifications

PPSQ-51A / 53A

benefits

- Amino acids can be sequenced accurately with extremely high data reliability.
- Ile and Leu residues with identical masses can be differentiated and the presence and position of S-S bonds can also be determined.
- Proteins can be analyzed directly, which makes operations extremely easy.

PPSQ-51A/53A Isocratic System

Specifications

Instrument	PPSQ-51A	PPSQ-53A
Reaction method	Edman degradation	
Reaction time	46.5 min/cycle	48 min/cycle
Number of reactors	1	3
Sample retention method	8 mm diameter glass fiber disc or PVDF membrane	
Reactor temperature control range	Room temp. +10 to 60 °C	
Converter temperature control range	Room temp. +10 to 70 °C	
Number of samples/solvents	7	
Sample/solvent supply method	N_2 gas pressure	
Dimensions	W 510 mm × D 500 mm × H 540 mm	
Weight	43 kg	45 kg
Elution method	Isocratic or gradient system	
Mobile phase	Special eluent specifically for Shimadzu protein fully-automated protein sequencers*	
Reaction reagent	Reagent specifically for Shimadzu protein fully-automated protein sequencers*	
Column	Column specifically for amino acid sequencing*	
Power requirement	Single-phase 120-230 V AC, 50/60 Hz, 1,500 VA max	
Nitrogen gas	Min. 99.9999 % purity	

* Available for purchase from Fujifilm Wako Pure Chemical Corporation.

Protein Primary Structure Analysis

Accurate Peptide N-Terminal Amino Acid Sequencing Using a MALDI-TOF MS Mass Spectrometer and Protein Sequencer

click here

Operating Principle and Features

Using the PPSQ sequencer to analyze an amino acid sequence using Edman degradation, as described on the previous page (p. 20), involves analyzing each amino acid one at a time, starting at the N-terminal. That eliminates mass or database dependence and other problems, but Edman degradation is not well suited to processing information for long sequences due to decreased reaction efficiency. To achieve more accurate and reliable N-terminal amino acid sequence information, combine Edman degradation data with In Source Decay (ISD) results obtained using a MALDI-TOF MS system.

Amino acid sequencing by mass spectrometry involves using the differences between fragment ion masses to determine the amino acid sequence of peptides. ISD increases the laser output to destabilize the substance being analyzed and break it into fragments. That results in obtaining a variety of fragments cleaved at the N-Cα bond in peptides (typically C-ions). Based on the data obtained, amino acid sequences are determined by either searching a database or by *De novo* sequencing. Database searching involves comparing the measured mass values to the database, which is the quickest and easiest method, but results depend on the data included in the database. In contrast, *De novo* sequencing does not use a database, though it does involve complicated data analysis that requires experience and proficiency.

Therefore, using software such as Mass++ can be helpful, because it eliminates the need to analyze data manually.

Results and Conclusion

Large amounts of information about peptides and proteins can be obtained from molecular weight data measured using a MALDI-TOF MS system. The molecular weight data is useful for quickly judging incorrect amino acid compositions and the presence of potential degradations or modifications. The accurate average molecular weight of peptides can be determined easily by selecting an appropriate matrix (Table 1). Even when using the MALDI-8020, a simple dedicated linear mode system, mass is detected precisely within 20 ppm of the theoretical molecular weight.

As shown in Table 2, N-terminal amino acid sequencing by either MALDI-TOF MS or Edman degradation provides a significant benefit for identifying amino acid sequences. Of all the methods currently available, N-terminal amino acid sequencing by Edman degradation remains the best method for determining the actual N-terminals of proteins and peptides. ISD also provides a reliable means of obtaining sequence information, but matrix interference generally prevents it from being used to observe low-mass fragments relevant to N-terminals. Fig. 2 shows results from BNP analysis using a combination of PPSQ and MALDI-8020 systems. Only a portion of the sequence can be determined using either one of these methods, but accurate sequence information can be obtained for the entire length by using both in a complementary way.

Measurement Method

B-type natriuretic peptide (BNP), a diuretic and vasodilatory hormone (Fig. 1) comprising 45 cyclic peptide residues, was used as the sample. To analyze the disulfide bonds that form the cyclic portions, the PPSQ sequencer requires reduction and alkylation, but MALDI-TOF MS enables direct analysis because samples can be reduced on the plate.

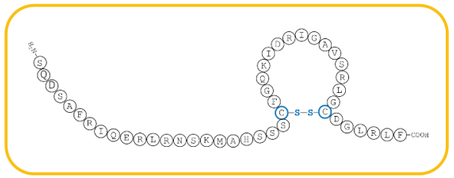


Fig. 1 Structure of Cyclic BNP Peptide

Table 1 Theoretical and Measured Masses for BNP

Peptide	Expected mass $[MH+]^+$	Measured mass $[MH+]^+$	Mass accuracy (ppm)
BNP	5038.6	5038.5	20

Table 2 Summary Table of Attributes Determined by PPSQ-50 Gradient System and MALDI-8020

Attribute	PPSQ-50 series	MALDI-8020
N-terminal sequencing	✓	
Internal, or C-terminal sequencing		✓
Differentiation of isobaric amino acids	✓	
Avoidance of databases	✓	
Ease of data interpretation (sequence)	✓	
Ease of use	✓	✓
Speed of analysis		✓
Intact mass determination		✓

Fig. 2 Determining BNP Sequence by Combination of Both ISD and Edman Degradation Sequencing

PPSQ-51A / 53A + MALDI-8020

benefits

- Obtain complete sequence coverage using PPSQ and MALDI-TOF MS systems in combination.
- Enables more reliable and accurate amino acid sequencing.
- MALDI-TOF MS enables direct analysis of cyclic peptides or peptides with blocked N-terminals.

PPSQ-51A/53A Gradient System

MALDI-8020

Specifications

Instrument	MALDI-8020
Mass range	*m/z* 1 to 500,000
Mass resolution	> 5,000 FWHM
Sensitivity	> 250 amol
Mass accuracy	< 20 ppm with internal calibration, < 150 ppm with external calibration
Acceleration voltage	15 kV
Laser	Solid-state laser
Wavelength	355 nm
Repetition frequency	50, 100, or 200 Hz (variable)
Flight distance	850 mm
Detector	Electron Multiplier
Ion source cleaning	Includes automatic cleaning functionality (depending on built-in solid-state laser)
Operating noise	< 55 dB
Main unit power supply	Single-phase 120 to 230 V AC, 50/60 Hz, 1,500 VA max
Dimensions	W 600 mm × D 745 mm × H 1,055 mm (excluding protrusions)
Weight	86 kg
Operating environment	Temperature: 18 to 28 °C Humidity: Max. 70 % (with no condensation)

Note: Refer to page 21 for details of PPSQ.

Analysis of Product Peptide Fragments

Using Integrated UHPLC System with High Repeatability for Mapping Peptides in Antibody Drugs

click here

Operating Principle and Features

Using HPLC for peptide mapping requires a system with high repeatability, because the analysis involves comparing elution profiles to confirm whether peptides are identical or have mutations. LC-2060 series integrated UHPLC systems are ideal for such analysis.

Measurement Method and Conditions

Samples were prepared by reduction, alkylation, and then trypsin enzyme digestion of human immunoglobulin G (IgG) (Fig. 1) and analyzed according to the analytical conditions in Table 1.

Results

The chromatogram from the trypsin-digested IgG shows that an extremely large number of peaks are detected and separated (Fig. 2). For peptide mapping, an extremely long gradual gradient is used to separate the many peaks. Consequently, results tend to have poor repeatability, especially if using a low-pressure gradient system. Therefore, the intra-day and inter-day repeatability were also checked. Key peaks (a to f) were selected from the chromatogram. Intra-day repeatability was calculated from six consecutive analysis results. Inter-day repeatability was calculated from the daily average values of three analyses on each of six days (Tables 2 and 3). Both the intra-day and inter-day repeatability values indicated good reproducibility.

Table 1 Analytical Conditions

Column:	Aeris PEPTIDE XB-C18 100 Å (150 mm × 2.0 mm I.D., 1.7 µm)
Mobile phase A:	0.1 % Trifluoroacetic acid in water
Mobile phase B:	0.08 % Trifluoroacetic acid in acetonitrile
Time Program: (B. Conc.)	0 % (0 min) → 45 % (90 min) → → 100 % (90.01 – 95 min) → → 0 % (95.01 – 110 min)
Flowrate:	0.2 mL/min
Column Temp.:	60 °C
Injection Volume:	10 µL
Detection:	PDA (215 nm)
Flow Cell:	High-speed high-sensitivity cell

Conclusion

LC-2060 series systems provide data with excellent repeatability even when using analytical conditions prone to cause poor repeatability in low-pressure gradient systems. They can also be connected to a mass spectrometer for peptide mapping.

Application Examples

- High-sensitivity analysis using a fluorescence detector
- Various UHPLC analyses

10 mg/mL Human IgG in water 20 µL
← 6 mol/L Guanidine hydrochloride in 0.25 mol/L Tris buffer (pH 7.5) 80 µL
← 0.5 mol/L Dithiothreitol in water 2 µL
Incubate at 37 °C for 30 min
← 0.5 mol/L Iodoacetamide in water 4.8 µL
Incubate at room temperature for 30 min in the dark
← 0.5 mol/L Dithiothreitol in water 2 µL
← 0.25 mol/L Tris buffer (pH 7.5) 700 µL
← 1 mg/mL Trypsin in 1 mmol/L HCl 4 µL
Incubate at 37 °C for 20 hours
← Trifluoroacetic acid 1 µL
Inject to UHPLC

Fig. 1 Pretreatment Process Flow

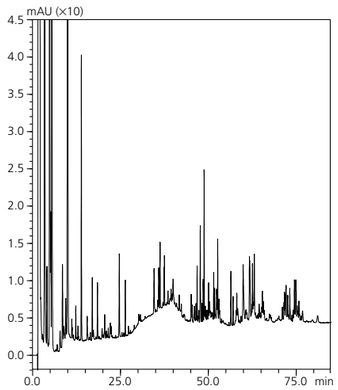


Fig. 2 Chromatogram of IgG Trypsin Digestion Products

Table 2 Intra-day Repeatability of Retention Times (n = 6)

Peak	Avg. R.T. (min)	Std. Dev. (min)	%RSD (%)
Peak a	9.929	0.027	0.271
Peak b	24.669	0.047	0.192
Peak c	36.299	0.042	0.117
Peak d	48.815	0.033	0.068
Peak e	59.864	0.032	0.054
Peak f	74.535	0.043	0.057

Table 3 Intra-day Repeatability of Retention Times (n = 6)

Peak	Avg. R.T. (min)	Std. Dev. (min)	%RSD (%)
Peak a	9.907	0.016	0.159
Peak b	24.708	0.033	0.132
Peak c	36.355	0.034	0.093
Peak d	48.877	0.034	0.093
Peak e	59.901	0.027	0.046
Peak f	74.555	0.036	0.049

LC-2060 Series

benefits

- Easy-access, front-panel interface ensures easy operation, even when wearing gloves.
- Can be connected to a mass spectrometer.
- Provides data with high repeatability.

Specifications

Instrument	LC-2060 series
Degassing unit	Five Lines: Mobile phase 4 + Rinse solution 1
Pumping method	Parallel-type double plunger
Pulsation	Max. 0.1 MPa (1.0 mL/min, 10 MPa, Water)
Flowrate setting range	0.0001 to 10 mL/min
Configuration	Four-solvent low-pressure gradient
Gradient settings	0 to 100 %, in 0.1 % steps
Maximum pressure	70 MPa 0.0001 to 3 mL/min
System delay volume	460 µL
Autosampler	
Injection method	Total-volume sample injection
Injection volume setting range	0.1 to 50 µL (Option: 0.1 to 100 µL, 1 to 500 µL, 1 to 2,000 µL)
Injection cycle time	Min. 14 sec (Specified condition)
Samples for processing	336 vials (1 mL), 216 vials (1.5 mL), 112 vials (4 mL), 4 sample plates
Sample cooler	4 to 45 °C
Column oven	
Heating and cooling method	Forced air circulation method
Containable column size	6 columns 10 cm long and 3 columns 30 cm long
Temperature control range	Room temperature - 12 to 90 °C, Setting range 4 to 90 °C
Flowrate switching valve	Max. 1 pc

Easily Determine Protein Secondary Structures

Analysis of Protein Secondary Structures

—Analysis on Changes of Secondary Structures in Egg White Proteins Caused by Thermal Denaturation—

click here

Operating Principle and Features

Multiple absorption peaks from C=O stretching vibration of peptide bonds overlap to appear as a broad peak near 1650 cm^{-1} (amide I band). Analyzing the peaks can provide information about the protein secondary structures. Each absorption band in the overlapping group of absorption bands can be determined by a curve-fitting process that optimizes peak information (position, intensity, and FWHM) for the curve being fit to each absorption band, so that the difference between the calculated and measured spectra is minimized. The calculated spectra are commonly based on the Lorenz or Gaussian curve fitting. The following describes the process for observing the secondary structural changes that occur due to thermal denaturation of proteins based on the second-derivative spectrum and peak separation.

Measurement Method

Egg white was used for the sample because it consists primarily of proteins. 60 µL samples were measured using a MicromATR measurement accessory with a heatable three-reflection ATR prism (diamond/ZnSe) installed. Since egg white hardens when heated, the three-reflection ATR prism was used because it can also be used to measure solid samples. Due to overlapping between amide I and water vapor peaks, the optical system was purged with dry air. Given the measurement conditions in Table 1, a temperature controller was used to increase the prism temperature from 40 to 100 °C in 10 °C steps, with each temperature setting held for two minutes after placing drops of egg white to ensure adequate heat transfer before measuring. To eliminate the effects of moisture in the egg white, analysis was based on difference spectra calculated by subtracting the spectrum for water at each temperature.

Table 1 Measurement Conditions

Resolution	4 cm^{-1}
Accumulation	100
Apodization function	Sqr-Triangle
Zero filling	4 times
Detector	DLATGS

Results and Discussion

The difference spectra between egg white and water at each temperature showed an increase in prominent peaks near 1625 cm^{-1} and 1675 cm^{-1} at 60 °C or higher temperatures (Fig. 1 is an enlargement of the 1700 to 1600 cm^{-1} area). That confirmed its correlation with thermal denaturation.

Conclusion

An FTIR spectrophotometer makes it easy to predict the changes in secondary structures due to thermal denaturation of proteins. It can contribute to protein modification technology, such as improving the thermal properties of proteins with a known structure by heating them to add structural mutations to structures that are prone to unfolding.

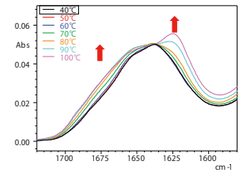


Fig. 1 Infrared Spectra of Amide I Band in Egg White

Analysis Using Second-Derivative Spectra

Evaluating second-derivative spectra can be helpful when investigating variations in the secondary structure of proteins (α-helix, β-sheet, β-turn, and random coil structures). The second-derivative spectrum (Fig. 2) determined from Fig. 1 confirmed that thermal denaturation was causing an increase in β-sheet structures near 1693 cm^{-1} and 1622 cm^{-1}, and β-sheet structures near 1637 cm^{-1} and α-helix structures near 1655 cm^{-1} to untangle. The peak shift due to thermal denaturation suggests the status of hydrogen bonds may have changed.

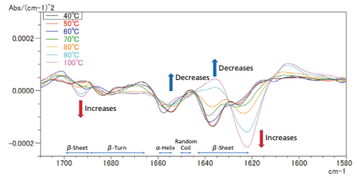


Fig. 2 Second-Derivative Spectra of Spectra in Fig. 1

Amide I Band Peak Separation

Based on the peak wavenumber and area value for each amide I band peak separated (measurement parameters in Table 2), secondary structures were attributed to each peak and the ratio of secondary structures was determined (Table 3). The resulting tendency for β-sheet structures to increase and α-helix structures to decrease matched the tendencies in the second-derivative spectrum.

Table 2 Conditions for Curve Fitting

Peak curve type	Gaussian function
Baseline	Offset 1 Pt
Range	1710 to 1580 cm^{-1}
Max. error	0.01%

Conditions for Curve Fitting

	α-helix	β-sheet	β-turn	Random coil
40 °C	30.3 %	37.9 %	16.4 %	15.4 %
100 °C	15.1 %	47.6 %	29.7 %	7.7 %

Application Examples (Shimadzu Application News No.)

- Predicting secondary structures in proteins
- Predicting the locations of mutations in proteins
- Evaluation of amyloid-β aggregation (A619)

IRTracer-100

benefits

- By using a heatable three-reflection ATR accessory, infrared spectra can be obtained from proteins in a heated environment.
- Slight variations in infrared spectral shapes can be shown clearly by calculating the second derivative of infrared spectra obtained.
- The secondary structures of proteins can be analyzed by separating amide I band peaks in second-derivative spectra.

Specifications

Instrument	IRTracer-100
Interferometer	Michelson interferometer (30° incident angle) Equipped with Advanced Dynamic Alignment system Sealed interferometer with Automatic Dehumidifier
Optical system	Single-beam optics
Beam splitter	Germanium-coated KBr for Middle IR (Standard) Germanium-coated CsI for Middle/Far IR (Optional) Silicon-coated CaF_2 for Near IR (Optional)
Light source	High-energy ceramic for Middle/Far IR (Standard) with 3 years guaranteed Tungsten lamp for Near IR (Optional)
Detector	DLATGS detector with temperature control for Middle/Far IR (Standard) MCT (Hg–Cd–Te) with liquid nitrogen cooling for Middle/Near IR (Optional) InGaAs for Near IR (Optional)
Wavenumber range	7,800 to 350 cm^{-1} (Standard) 12,500 to 240 cm^{-1} (Optional)
Resolution	0.25, 0.5, 1, 2, 4, 8, 16 cm^{-1} (Middle/Far IR) 2, 4, 8, 16 cm^{-1} (Near IR)
Dimensions	W 600 mm × D 665 mm × H 295 mm
Weight	47 kg
Measurements	Spectrum measurement, continuous measurement, atmospheric correction measurement, continuous measurement using ASC, simple measurement mode

Glycan Analysis

Analysis of Glycans by HPLC Detection of Fluorescence-Marked Glycans

click here

Operating Principle and Features

Glycans can affect the safety and efficacy of biopharmaceuticals. One technique used to analyze glycans is to mark them with fluorescence and then analyze them by HPLC using a fluorescence detector. Shimadzu RF-20Axs fluorescence detectors offer low noise and good S/N levels (compared to previous models, as shown in Fig. 1) to provide excellent sensitivity and linearity for glycan analysis. Glycan fluorescent labeling methods include those using pyridylamino (PA)-glycan and 2-aminobenzamide (2-AB)-labeled glycan. Either type of fluorescent-labeled glycans can be analyzed in the same manner.

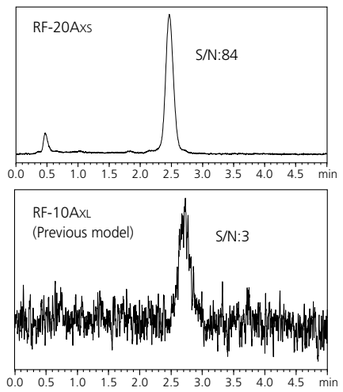


Fig. 1 Chromatograms of 10 fmol PA-Glycan

Table 1 Analytical Conditions

Column:	Aeris PEPTIDE XB-C18 (150 mm × 2.1 mm I.D., 1.7 µm)
Mobile phase A: (pH 4.5)	20 mmol/L Ammonium Formate 0.0095 % (v/v) Formic acid-water
Mobile phase B:	20 mmol/L Ammonium Formate 0.0095 % (v/v) Formic acid-Methanol
Time Program (B. Conc.):	0 % (0 min) → 5 % (60 min) → → 10 % (70 min) → → 100 % (70.01 min- 80 min) → → 0 % (95.01 – 110 min)
Flowrate:	0.4 mL/min
Column Temp.:	40 °C
Injection Volume:	3 µL
Detection:	RF-20Axs (Ex: 320 nm, Em: 400 nm)

Measurement Method and Conditions

Glycans in an antibody drug were analyzed by HPLC with detection by the high-sensitivity RF-20Axs fluorescence detector. An Aeris PEPTIDE XB-C18 core-shell analytical column was used. The column packing material penetration was optimized for analyzing peptides and other macromolecules, which makes it effective for separating glycans and contaminants in antibody drugs.
Two types of antibody drugs were treated with trypsin and Glycopeptidase F was used to cleave glycans. Then the glycans were fluorescently derivatized by PA and used for analysis (Table 1).

Results

Peak differences noticed between the chromatograms for antibody drugs A and B after about 50 minutes of elution (*) clearly indicated the drugs contained different glycan levels. In addition, many peaks with different response levels were observed (Fig. 2 and 3).

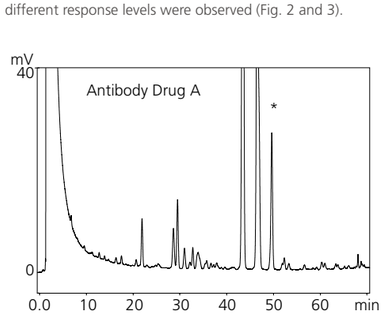


Fig. 2 Chromatogram of PA-Glycans from Antibody Drug A

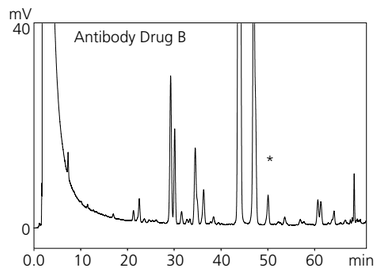


Fig. 3 Chromatogram of PA-Glycans from Antibody Drug B

Conclusion

Glycans in antibody drugs can be analyzed using HPLC by fluorescent labeling the glycans after trypsin digestion. RF-20Axs detectors offer high sensitivity and low noise. They can also be connected to an LC-2060 series integrated HPLC system (refer to p. 24).

Application Examples (Shimadzu Application News No.)

- Analysis of 2-AB glycans (L483)
- Quantitative analysis of favipiravir spiked in plasma (L570)

This analysis of glycans in antibody drugs was achieved with help from professor Kenichiro Tadoroki of the Laboratory of Analytical and Bio-Analytical Chemistry, School of Pharmaceutical Sciences, University of Shizuoka.

RF-20Axs

benefits

- The low noise and excellent S/N ratio ensure ample sensitivity for glycan analysis.
- Cell temperature control functionality enables highly reproducible data acquisition.
- Standard, semi-micro, inert, and other cells can be selected based on the given analysis.

Specifications

Instrument	RF-20Axs fluorescence detector		
Light source	Xenon lamp, low-pressure mercury lamp (To check wavelength accuracy)		
Wavelength range	Excitation wavelength from 200 to 900 nm, Fluorescence wavelength from 200 to 900 nm		
Cell temperature control range	(Room temperature - 10 °C) to 40 °C		
Cell	Standard conventional cell	Volume: 12 µL	Pressure capacity: 2 MPa
	Optional semi-micro cell	Volume: 3 µL	Pressure capacity: 2 MPa
	Optional inert cell	Volume: 12 µL	Pressure capacity: 2 MPa
Sampling rate	Max. 100 Hz (1 wavelength mode)		
Function	Four-wavelength detection, wavelength scanning		
Operating environment	4 to 35 °C		
Dimensions	W 260 mm × D 500 mm × H 210 mm		
Weight	18 kg		
Power requirement	100 to 240 V AC, 400 VA, 50/60 Hz		

Glycan Analysis

N-Linked Glycan Analysis Using MALDImini-1
Structural Analysis and Identification of Sialyl Linkage Isomers
click here

Operating Principle and Features

Conventional MS^n mass spectrometers are large and require peripheral equipment, but the space-saving MALDImini-1 fits in a space smaller than a piece of A3 size paper. The built-in vacuum pump means the system can be operated anywhere regular 100 V AC power is available. An optional kit is also available for supplying gas from small gas cartridges. Additionally, the MALDI ion source and Digital Ion Trap (DIT) technology enable high-sensitivity MS and MS^n measurements across a wide mass range, even for trace sample quantities.

Measurement Method

Proteins include many acidic glycans that contain sialic acids, which are analyzed by an HPLC or a mass spectrometer. HPLC generally requires using a reference glycan preparation and can have difficulty discriminating between complex glycans down to sialic acid linkages, for example. Mass spectrometers can have problems with unstable sialic acid residues being prone to desorption during analysis and an inability to discriminate between forms with different binding isomers. Therefore, the sialic acid residues on *N*-linked glycans derived from serum were stabilized using the sialic acid linkage specific alkylamidation method (SALSA method*1 in Fig. 1) developed by Shimadzu. The compact MALDImini-1 MALDI-DIT mass spectrometer was used for detection and analysis. The SALSA method generates a mass difference between linkage forms using a two-stage reaction that amidates α2,6-linked sialic acids with isopropylamine (iPA) and amidates α2,3-linked sialic acids with methylamine (MA). That means MS can be used to discriminate between sialic acid linked isomers that otherwise would have identical masses.

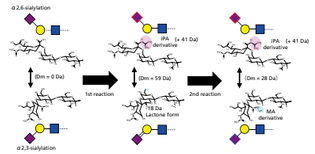


Fig. 1 Overview of Sialic Acid Linkage Specific Alkylamidation (SALSA) Method

5 µL of commercial serum were denatured and reduced by SDS and DTT. *N*-linked glycans were cleaved from glycoproteins by adding PNGaseF (Peptide-*N*-glycosidase F) and letting it react for 18 hours at 37 °C. 4 µL of the cleaved *N*-linked glycans were mixed directly with 20 µL of the SALSA reaction solution and left to react for one hour at room temperature. Later, a stabilizer reagent with a lactonic structure was added and mixed, and then the GL-Tip Amide (GL Sciences) was used to remove the excess reagent. Also, the reducing terminal of the glycan was labeled with 2-aminobenzamide. Samples prepared by the process above were dripped onto a 0.5 µL sample plate and 0.5 µL of a matrix (α-cyano-4-hydroxycinnamic acid solution containing sodium chloride) was layered on top and dried. Then the MALDImini-1 was used for MS^n analysis.

Results

A wide variety of bifurcated, trifurcated, and other mainly glycan composites were detected from the *N*-linked glycans derived from serum glycoproteins (Fig. 2). A comparison of two types of MS^2 spectra for trifurcated glycans shows the glycans were detected 28 Da apart, which infers that there are two different glycans (α2,3- and α2,6-linked forms) in the same location. Also, given that MS^2 results show a neutral loss mass equivalent to modified sialic acids, which is the basis for differentiating between sialic acid linkage forms, presumably the peak at *m/z* 3117.1 indicates a mixture of α 2,3-/α 2,6- forms and *m/z* 3145.2 indicates only the α2,6- form. MS^3 analysis was used to determine the location on the glycan that generated the fragment ion. For example, the fragment ion at *m/z* 720.0 in the MS^2 results for a biantennary glycan at *m/z* 2448.1 cannot be explained by successive desorption of glycans from the non-reducing terminal. However, a comparison of MS^3 results for fragment ions that include sialic acid (*m/z* 2107.0) and do not include sialic acid (*m/z* 1783.9) indicates that the *m/z* 720.0 fragment ion is not detected in the latter results. That means the *m/z* 720.0 fragment ion is derived from the three glycans on the non-reducing terminal that includes the sialic acid.

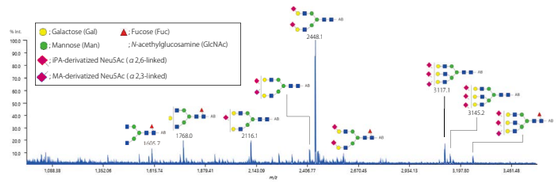


Fig. 2 Mass Spectrum of *N*-Linked Glycans Derived from Serum Glycoproteins

Conclusion

Stabilization of sialic acids by the SALSA method and MS^n analysis by the MALDImini-1 system can be used to analyze the structure of glycans, including the sialic acid linkage types.

Application Examples (Shimadzu Application News No.)

- Protein identification
- Structural analysis of glycans and glycopeptides (B100)
- Checking the mass of various molecules

*1 Patent No. 06135710

MALDImini-1

benefits

- Compact size and simple configuration allows installation in confined spaces.
- Samples can be measured immediately at the same location they are prepared.
- Suitable for a wide range of applications, from measuring the molecular weights of trace samples to structural analysis of complex molecules.

Specifications

Instrument	MALDImini-1	
Mass range	*m/z* 650 to 70,000	
MS/MS mass range	*m/z* 350 to 5,000	
Mass resolution	> 4000 FWHM, [Glu1]-Fibrinopeptide B *m/z* 1570.68, scan speed 1000 Da/s	
Sensitivity (MS)	1 fmol ([Glu1]-Fibrinopeptide B *m/z* 1570.68) 500 fmol (BSA *m/z* 66,431)	
Sensitivity (MS/MS)	10 fmol ([Glu1]-Fibrinopeptide B *m/z* 1570.68)	
Mass accuracy	Internal standard: < 200 ppm	External standard: < 200 ppm (*m/z* 1,000 to 5,000)
MS^n	1 ≤ n ≤ 3	
Laser	Medium: Nd:YLF	Wavelength: 349 nm
Sample plate	Disposable FlexiMass-DS and stainless steel FlexiMass-SR (26 × 76 mm)	
Gases	Argon and helium (min. 99 % at 40 to 60 kPa)	
Gas cartridge	Regulator, He gas tubing, Ar gas tubing, and gas cartridge holder	
Power supply	AC 100 to 240 V, 50/60 Hz, 960 VA	
Dimensions	W 309 mm × D 385 mm × H 320 mm	
Weight	25 kg	
Operating environment	Temperature: 18 to 26 °C	Humidity: 40 to 70 % max. (with no condensation)
Software	Saving data:	Database using SQLite
	Export file formats:	mzML and mzXML

Glycan Analysis

Glycosylation Profile of IgGs Using a Linear Benchtop MALDI-TOF MS and Affinity Purification of Fc

click here

Operating Principle and Features

The MALDI-8020 is a linear-mode MALDI-TOF mass spectrometer with a small installation footprint. It is typically used for quality control or profiling applications for peptides, proteins, polymer or oligonucleic acids, and other substances. Despite the benchtop design, the ion optical system features a large diameter inlet to ensure proper system performance levels are maintained for long periods and to reduce the risk of ion source contamination. The UV laser-based rapid automatic ion source cleaning function (TrueClean) can clean the ion extractor electrode without releasing the vacuum pressure. The system can manage all data and other information in one location and includes a tool for assisting with strict compliance with FDA 21 CFR Part 11.

Measurement Method

Especially for biopharmaceutical development applications, high-end mass spectrometers are commonly used due to the extreme importance of evaluating the properties of *N*-/*O*-linkages. However, for batch analysis intended for screening or QA/QC applications, relatively inexpensive and user-friendly commercial systems are preferred. The MALDI-8020 model fits this need, offering more than adequate specifications for general profiling, high throughput, and an excellent value for the price.

The following describes an example of profiling IgG glycan modifications without releasing any glycans. Human $IgG1_K$ monoclonal antibodies (NISTmAb), IgG from mouse serum, and myeloma IgG were dissolved in a Tris-NaCl buffer solution and incubated together with IdeZ (IgG-degrading enzyme) for two hours at 37 °C. Then Protein A magnetic beads were used to recover Fc sections. The Fc regions were eluted from the beads with an acidic solution and desalted with ZipTip C18 tips. Sinapic and ferulic acids were respectively dissolved in a 50 % acetonitrile solution containing 0.1 % TFA to a final concentration of 20 mg/mL. Then the resulting solutions were used as matrices for MS analysis.

Results

In the mass spectrum of IgG full-length, the IgG molecular weight was observed near 150 kDa (Fig. 1). Due to inadequate mass resolution in the *m/z* range for large molecular weights, the mass spectrum for about 25 kDa of the Fc region modified by a glycan was analyzed (Fig. 2). The mass gap between peaks in each spectrum indicates one sugar unit. Furthermore, MS measurements of each Fc region were repeated three times and statistically analyzed using eMSTAT Solution. A score plot was obtained easily by simply making some minimal adjustments to a few parameters (Fig. 3).

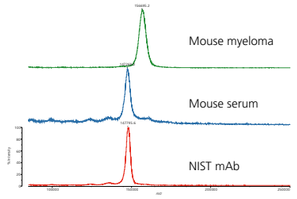


Fig. 1 Mass Spectrum of IgG Full-length

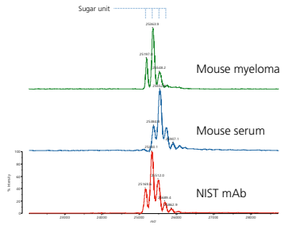


Fig. 2 Mass Spectrum of Fc Region

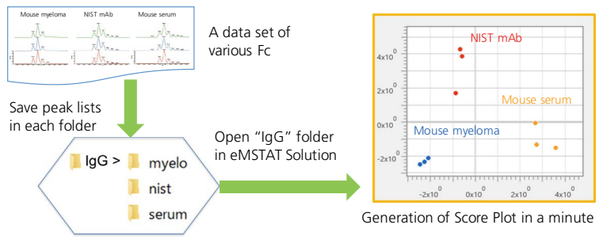


Fig. 3 Classification of Fc Region Using eMSTAT Solution

Conclusion

The MALDI-8020 provides ample MS resolution for recognizing three Fc regions with different varieties of glycan modifications. In addition, statistical analysis using eMSTAT Solution enabled quick classification of the three Fc types, which could be used for batch analysis, QA/QC, or other applications.

Application Examples

- Quality control of antibody drugs
- Synthesis confirmation of nucleic acid drugs

MALDI-8020

benefits

- Enables rapid benchtop glycan profiling with minimal pretreatment.
- Easy maintenance and low running costs
- Ideal for analyzing nucleic acids, proteins, and even polymers.

Specifications

Instrument		MALDI-8020
Mass range		*m/z* 1 to 500,000
Mass resolution		>5,000 FWHM
Sensitivity		>250 amol
Mass accuracy		<20 ppm with internal calibration, <150 ppm with external calibration
Acceleration voltage		15 kV
Laser		Solid-state laser wavelength: 355 nm Repetition frequency: 50, 100, or 200 Hz (variable)
Flight distance		850 mm
Detector		Electron Multiplier
Ion source cleaning		Includes automatic cleaning functionality (depending on built-in solid-state laser)
Sample plate		Disposable FlexiMass-DS and stainless steel FlexiMass-SR
Operating noise		<55 dB
Main unit power supply		Single-phase 100 to 240 V AC, 50/60 Hz, 1 kVA
Dimensions		W 600 mm × D 745 mm × H 1,055 mm (excluding protrusions)
Weight		86 kg
Operating environment		Temperature: 18 to 28 °C Humidity: Max. 70 % (with no condensation)
Data analysis software		eMSTAT Solution
Data analysis functionality	Univariate analysis	t-Test, Mann-Whitney U-Test, ANOVA (analysis of variance)
	Multivariate analysis	PCA (principal component analysis), PLS-DA
	Discriminant analysis	Support Vector Machine (SVM), Random Forest
	Other	Dynamic grouping
Display functionality	Multivariate analysis	Peak Matrix, Box Plot, ROC, AUC, Score/Loading Plot, Dendrogram
	Discriminant analysis	Discriminant analysis results (Group, Score) superimpose points for unknown samples on a score plot
Input/output data	Input	Peak list (ASCII, JCAMP, or mzML format)
	Output	Peak list (txt format), data analysis results (xlsm format), graph screenshot

Quantitation of Proteins

Quantitation of Proteins Using BioSpec-nano

Operating Principle and Features

The BioSpec-nano has two available optical path lengths, 0.2 mm and 0.7 mm, and can quantify proteins and nucleic acids in sample quantities as low as 1 µL. Samples can also be measured using an optional cell with a 5 mm optical path length (for 2 mL sample quantities). That means only a small sample quantity is needed for quick protein concentration measurements or to check spectra.
A wiping mechanism enables automatic cleaning between samples, ensuring extremely low carryover and reducing inconsistencies that may occur with manual cleaning (Fig. 1).

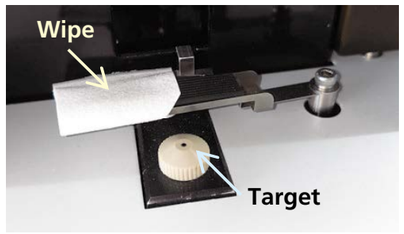


Fig. 1 Automatic Wiping Function

Measurement Method

Measurement samples were prepared by diluting bovine immunoglobulin (IgG) to 1000 µg/mL with phosphate buffered saline. With the optical path length set to 0.7 mm (Fig. 2), 4 µL of the sample was dripped onto the target to measure the optical density (OD) at 280 nm. The protein concentration is calculated based on the molar absorption coefficient (ε = 280) and molecular weight values entered in the software's analytical condition selection window (Fig. 3). The 280 ε value can also be calculated by the software if the number of tryptophan, tyrosine, and cysteine residues in the amino acid sequence is entered.

Fig. 2 Optical Path Length Setting Area

Conclusion

Using the BioSpec-nano, sample concentrations can be measured from small sample quantities of 1 to 4 µL by simply dripping the sample onto the stage. That eliminates any need for any manual processes, such as raising/lowering an arm, placing a cell in position, or wiping off the sample after each measurement. In addition to concentration determination, OD values can be displayed for any user-specified wavelength.

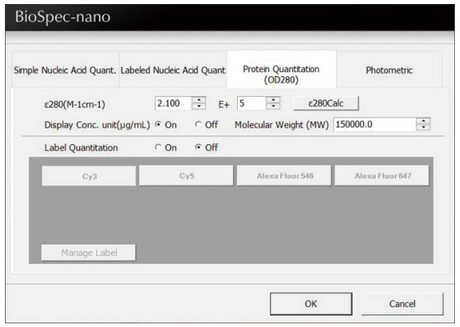


Fig. 3 Analytical Condition Selection Window

Results

Measurement results are shown in Fig. 4. The results show a concentration of approx. 1000 µg/mL, which is the same as prepared. Sample concentration values and spectra can be confirmed in the detail display window. Results can be output in either CSV or PDF format.

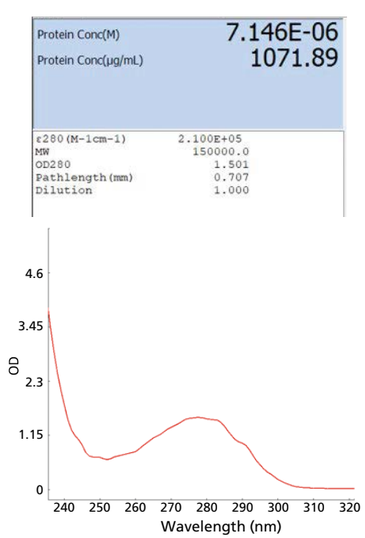


Fig. 4 Analytical Results for 0.7 mm Optical Path Length

Application Examples

- Measuring single- or double-stranded DNA concentrations (refer to p. 6)
- Measuring labeled nucleic acid concentrations
- Measuring RNA concentrations

BioSpec-nano

benefits

- Measure the concentration of proteins or check the purity.
- Measure sample quantities as small as 1 µL.
- Achieve low carryover with the automatic wiping function.

Specifications

Instrument	BioSpec-nano
Wavelength range	220 to 800 nm
Spectrum bandwidth	3 nm
Wavelength accuracy	±1 nm
Pathlength	0.2 mm, 0.7 mm
Photometric value unit	OD (Optical Density), absorbance converted with 10 mm pathlength
Sample volume	1 µL min. (pathlength: 0.2 mm) 2 µL min. (pathlength: 0.7 mm)
Light source	Xenon flash lamp
Monochromator	Holographic grating
Detector	Photo diode array
Auto wiping function	Provided
Spectrum measuring time	3 sec
Quantitation range	Pathlength 0.2 mm, 1 to 75 OD, 50 to 3,700 ng/µL Pathlength 0.7 mm, 0.3 to 21 OD, 15 to 1,000 ng/µL Optional 5 mm pathlength cell, 0.04 to 3 OD, 2 to 150 ng/µL
Dimensions	W 210 mm x D 214 mm x H 417 mm
Weight	7 kg
Analysis mode	Simple nucleic acid quantitation, labeled nucleic acid quantitation, protein quantitation, labeled protein quantitation, photometric measurement

Note: The droplet formation status will affect analysis results. Measure quantities that are large enough to enable proper droplet formation.

Characterization of Monoclonal Antibodies

Molecular Weight Analysis of Monoclonal Antibodies Using the LCMS-9030 Quadrupole Time-of-Flight Mass Spectrometer

click here

Operating Principle and Features

The LCMS-9030 is a Q-TOF mass spectrometer that includes both quadrupole and time-of-flight separation systems, two types of mechanisms for separating ions. The system includes unique Shimadzu technologies in a variety of locations for acquiring data with both high sensitivity and high resolution, while also ensuring mass accuracy is always stable. For example, it includes technology for increasing the ion usage rate, machining technologies for manufacturing powerful and finely detailed grating electrodes, technology for precision temperature control, and technology for optimizing the distribution of electric potential values.

Analytical Conditions

Human IgG1$_K$ monoclonal antibodies (mAb) were dissolved in 50 mmol/L aqueous ammonium hydrogen carbonate solution to prepare a 1 mg/mL standard solution (intact mAb). mAb subunits were prepared by adding 8 mol/L urea and a 50 mmol/L Tris-HCl buffer solution containing 50 mmol/L DL-dithiothreitol to 100 µg of the intact mAb to reduce the antibodies to H and L-chains. Measurement conditions for the intact mAb and mAb subunits are indicated in Table 1.

Table 1 Analytical Conditions

[LC]			
Column:		Restek C4 (150 mm × 2.1 mm I.D., 5 µm)	
Column oven:		50 °C (Intact), 85 °C (subunits)	
Solvent A:		0.1 % formic acid/water	
Solvent B:		0.1 % formic acid/acetonitrile	
Intact	Gradient: (Conc. B)	0 % (0.5 min) → 5 % (3.0 min) → → 60 % (5.5 – 5.6 min) → 5 % (10 min)	
Subunits	Gradient: (Conc. B)	0 % (0.5 min) → 15 % (1.5 min) → → 30 % (2.5 – 3.5 min) → → 45 % (7.5 – 8.5 min) → → 50 % (10.5 – 10.6 min) → → 15 % (15 min)	
Flowrate:		0.4 mL/min	
[Q-TOF]			
Mode:		MS mode	
		Intact	Subunits
TOF Start *m/z*:		1000.0000	800.0000
TOF End *m/z*:		4000.0000	4000.0000
Event Time (s):		5.0	2.0
Pulser Inj. Times:		9993	3993
Interface:		300 °C	
Nebulizer gas:		3 L/min	
Drying gas:		10 L/min	
Heating gas:		10 L/min	

Results

Fig. 1 shows the TIC chromatogram, deconvoluted MS spectrum, and the MS spectrum measured from the intact mAb. A comparison of the mass values measured from the intact mAb to theoretical mass values confirmed that measured values were consistent with theoretical values to a precision level of 25 ppm or less (Table 2). Protein Metrics Intact Mass Workflow software was used for the deconvolution of intact mAb. The TIC chromatogram, MS deconvoluted spectrum, and MS spectrum were also similarly analyzed for mAb subunit H and L-chains. Results from checking their molecular weights provided good values.

Table 2 Comparison of Measured and Theoretical Mass Values

		Sample name	NIST mAb
		Peak#	1
Name	Expected mass	Mass	
G2F/G2F+Hex	148848	148850	1.8
G2F/G2F	148686	148688	1.9
G1F/G2F+Lys	148652	148653	1.6
G1F/G2F	148524	148525	1.0
G1F/G1F+Lys	148490	148489	-0.9
G1F/G1F	148362	148362	0.7
G0F/G1F-GluNAc	147996	148000	3.5
G0F/G1F+Lys	148328	148326	-1.4
G0F/G1F	148199	148200	0.7
G0F/G0F-GlcNAc	147834	147837	3.2
G0F/G0F-2GlcNAc	147631	147631	0.0
G0F/G0F+Lys	148165	148164	-1.5
G0F/G0F	148037	148039	2.0

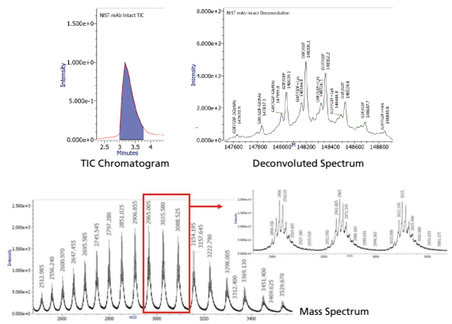


Fig. 1 NIST mAb Intact Results

Conclusion

Using the LCMS-9030 Q-TOF mass spectrometer with Protein Metrics software enables evaluation of molecular weights in biopharmaceuticals with high sensitivity and high resolution to achieve consistently high precision.

Application Examples

- Quantitative analysis of pharmaceuticals
- Identification of impurities
- Peptide mapping

LCMS-9030

benefits

- Trace quantities of impurities contained in pharmaceutical ingredients can be measured with high sensitivity.
- Accurate mass information can be used to identify impurities in products.
- Deconvoluted spectra can be used to check the molecular weights in antibody drugs.

Specifications

Instrument		LCMS-9030
Mass range		Quadrupole mass range: *m/z* 10 to 2,000
		TOF mass range: *m/z* 10 to 40,000
Sensitivity	ESI positive	1 pg reserpine
		S/N > 1,000:1 (RMS) in MS mode
		S/N > 10,000:1 (RMS) in MS/MS mode
	ESI negative	1 pg chloramphenicol
		S/N > 1,000:1 (RMS) in MS mode
		S/N > 10,000:1 (RMS) in MS/MS mode
Resolution (Quadrupole)		R < 0.8 u FWHM
Resolution (TOF)	ESI positive	30,000 FWHM at *m/z* 1,972
	ESI negative	30,000 FWHM at *m/z* 1,626
Mass accuracy	MS mode	< 1 ppm (peak to peak) at *m/z* 622.5662, NaI cluster (internal calibration)
	MS/MS mode	< 2 ppm (peak to peak) at *m/z* 1072.2489 > 472.6719 NaI cluster (external calibration)
Mass accuracy temperature stability		1 ppm/24 h, 18 to 28 °C at constant temperature
Maximum acquisition rate		100 Hz
Polarity switching time		1 sec
Interface		Standard: ESI
		Optional: APCI, DUIS, CDS, Nano-ESI

Measuring Protein Aggregates (with temperature control and mixing)

Accelerated Testing of Protein Stability Using the Aggregates Sizer TC (With Temperature Control)

click here

Operating Principle and Features

The Aggregates Sizer aggregation analysis system for biopharmaceuticals can measure all aggregates within the previously difficult-to-measure 0.1 to 10 µm range at the same time, measure aggregates while applying a stress, and perform quantitative measurements. The Aggregates Sizer is a particle size analyzer that measures particle diameters based on the laser diffraction method. Normally, using the laser diffraction method, particle size can be determined based on a sample's scattering patterns but concentration cannot be determined. However, the Aggregates Sizer can measure absolute concentration values by calibrating with a standard sample of known particle sizes and concentrations.

Measurement Method and Conditions

The sample solution was prepared by mixing freeze-dried bovine gamma globulin with PBS (pH 7.4) to a concentration of 1 mg/mL. 5 mL of the sample solution was measured in a temperature-controlled batch cell (Fig. 1) while stirring for 40 minutes at 190 strokes per minute. Stirring rods made of three materials, PEEK, stainless steel, and glass, were used for comparison. A temperature-controlled circulator was used during accelerated testing to maintain the temperature at three different constant temperature levels (23, 30, and 42 °C) for comparison.

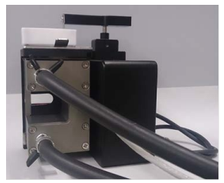


Fig. 1 Batch Cell (with temperature control function)

Results

A comparison of aggregate formation during the 40 minutes at 23 °C for the 0.2 to 2 µm range versus the 2 to 10 µm range is shown in Fig. 2. It indicates that aggregate formation was greatest in the 2 to 10 µm range using the PEEK stirrer, and greatest in the 0.2 to 2 µm range using the stainless steel stirrer. The glass stirrer resulted in the least aggregate formation in both ranges. The time-course changes in the particle size distribution for the PEEK stirrer at 42 °C (Fig. 3) show that aggregate formation occurs as a function of time. It also confirms that aggregate formation depends on temperature.

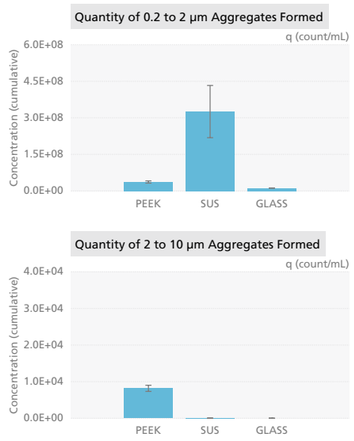


Fig. 2 Comparison of Aggregate Formation in Each Size Range Using Different Stirring Rod Materials (after 40 minutes at 23 °C)

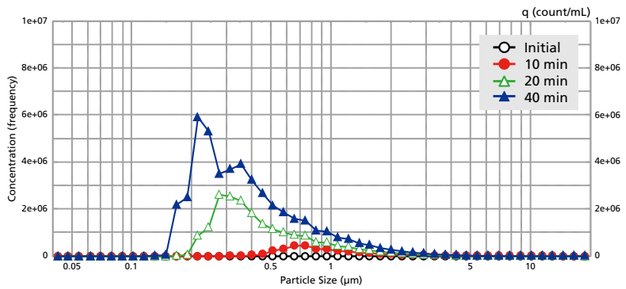


Fig. 3 Aggregate Formation Process during Accelerated Testing (with PEEK at 42 °C)

Conclusion

The Aggregates Sizer is ideal for evaluating protein stability, because it is able to measure aggregate concentrations in real time as stirring stress is applied under temperature-controlled conditions and as the materials that contact liquid are varied.

Application Examples (Shimadzu Application News No.)

- Evaluating the stability and responsiveness to stress of biopharmaceuticals
- Considering additives for inhibiting aggregation (Q117)
- Evaluating the concentration of particles in blood (Q120)

index

Aggregates Sizer

benefits

- With a single system, it is possible to measure all aggregates within the 0.1 to 10 µm range at the same time.
- Real-time measurements can be performed while controlling the temperature and applying stirring stress.
- Micro quantities (125 µL) can be measured using a micro cell.

Specifications

Instrument	Aggregates Sizer
Measurement principle*1	Laser diffraction method
Measurement range	Particle size range: 100 nm to 10 µm (Size distribution displayed: 40 nm to 20 µm)
Concentration measurement accuracy*2	± 30 or less
Concentration range*3	Particle size 100 nm: 2 µg/mL to 12 µg/mL Particle size 1 µm: 0.5 µg/mL to 10 µg/mL Particle size 10 µm: 10 µg/mL to 180 µg/mL
Batch cell	Cell material: Quartz glass Required liquid volume: 5 mL Stirring mechanism: Up-and-down movement of blade Stirring plate material: Stainless steel, glass, PEEK Temperature range with temperature control function: 20 to 42 °C ± 1 °C, set from PC Operating environment: Temperature: 10 to 30 °C Humidity: 20 to 80 % (with no condensation)
Micro cell	Material: Quartz glass and PTFE (cell cap with temperature control function) Required liquid volume: Approx. 0.125 mL Temperature range with temperature control function: 20 to 42 °C ± 1 °C, set from PC Operating environment: Temperature: 10 to 30 °C Humidity: 20 to 80 % (with no condensation)
Dimensions and weight	Approx. W 680 mm × D 280 mm × H 430 mm, and approx. 31 kg

*1 The measurement range depends on particle characteristics, such as shape.
*2 Concentration measurement accuracy values were measured using reference samples and procedures specified by Shimadzu.
*3 The concentration range depends on particle characteristics, such as shape.

index

Evaluating Aggregates in Protein Drug Products

Characterization of Insoluble Subvisible Particles in Biopharmaceuticals Using the Flow Imaging Method

click here

Operating Principle and Features

For protein drug evaluation, the United States Pharmacopeia and Japanese Pharmacopoeia specify using the light obscuration (LO) method to evaluate insoluble particles that are 10 µm or larger. Meanwhile, flow imaging (FI), a dynamic image analysis method that offers high sensitivity for particles with high permeability and also the ability to classify particles in images, has been gaining attention as a method for analyzing micrometer-level insoluble subvisible particles.
The iSpect DIA-10 dynamic particle image analysis system is used to acquire images of particles in liquid samples for measuring the size distribution, concentration, and shapes of particles based on the flow imaging method. Due to the small sample quantities used (minimum 50 µL for measurements) and the optical system that misses very few particles, it is ideal for evaluating insoluble subvisible particles in biopharmaceuticals.

Measurement Method and Conditions

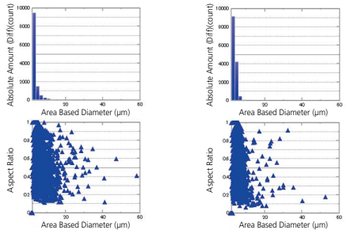
Sample solutions were prepared using freeze-dried human immunoglobulin. The sample powder was dissolved in a pH 5.0 citrate-phosphate buffer solution (1 mg/mL), which was filtered through a 100 nm filter and the filtrate was used as the sample solution.
Half the sample solution was heated for three minutes at 80 °C. The other half was stirred with a PEEK polymer stirring plate for ten minutes. Then the heat-aggregated and the stirring-aggregated samples were measured according to the conditions in Table 1.

Table 1 Measurement Conditions

Frame rate	8 frame / sec
Efficiency	97 %
Sample amount	50 µL
Threshold	220
Flowrate	0.1 mL / min

Results

The particle size distributions and scatter plots obtained from the measurements are shown in Fig. 1. Particle images are shown in Fig. 2. The particle images can be used to distinguish between aggregates, air bubbles, and oil droplets. Particle concentration measurement results are shown in Table 2 and Fig. 3.
Though it is difficult to analyze the shape of particles smaller than 5 µm, detecting them is possible.



a) Heat Aggregate Sample b) Stirring Aggregate Sample

Fig. 1 Particle Size Distribution and Scatter Diagrams

Table 2 Observed Particle Count and Number Concentration

			Heat aggregate sample	Stirring aggregate sample
Observed particle count	Overall		32246	18813
	By size	< 2 µm<	20129	4669
		2 - 10 µm	11797	14057
		10 - 25 µm	298	78
		≥ 25 µm	22	9
Particle concentration* (count/mL)	Overall		668102	389784
	By size	< 2 µm	417051	96737
		2 - 10 µm	244421	291246
		10 - 25 µm	6174	1616
		≥ 25 µm	456	186

* Particle concentration is calculated from the observed particle count, volume of area observed, and number of images

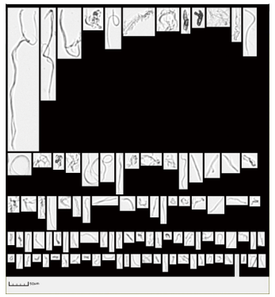


Fig. 2 Particle Image of Aggregates from Stirring

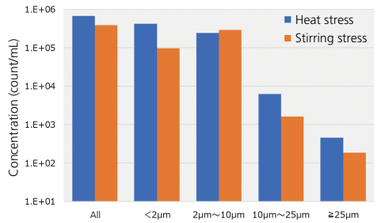


Fig. 3 Count Concentration for Respective Particle Sizes

Conclusion

iSpect DIA-10 systems can measure very small quantities of samples with excellent imaging efficiency. Even the type of insoluble subvisible particles can be predicted from particle images, making it ideally suited for evaluating the concentration of micrometer-level insoluble subvisible particles contained in biopharmaceuticals.

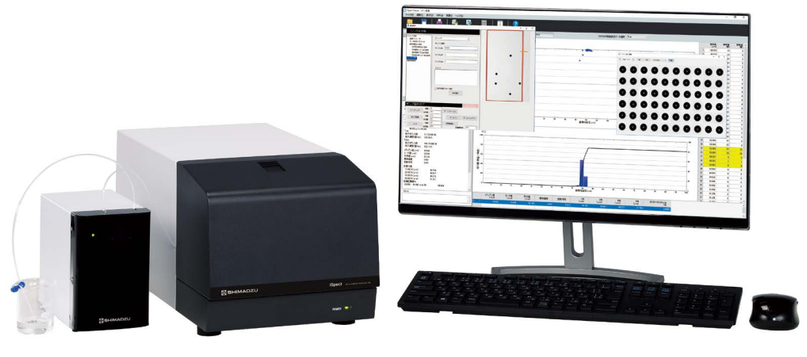
Application Examples (Shimadzu Application News No.)

- Evaluating the concentration of insoluble particles in biopharmaceuticals
- Evaluating contaminants, coarse particles, and particle shapes in pharmaceuticals
- Evaluating the size of suspended particles in eye drops (Q122)

iSpect DIA-10

benefits

- The optical system, which overlooks very few particles, can be used to evaluate the particle count concentration of micrometer-level aggregates.
- Sample quantities as small as 50 µL can be measured.
- Simple measurements can be performed in three steps, which minimizes the burden on operators.



Specifications

Instrument	iSpect DIA-10	
Measurement method	Dynamic image analysis method	
Particle size measurement range*1	5 to 100 µm	
Particle count concentration reproducibility*2	CV ≤ 5 %	
Measurement parameters	Particle size Area circle equivalent diameter, perimeter equivalent diameter, maximum length, maximum perpendicular length, vertical Feret diameter, horizontal Feret diameter, particle perimeter, envelope perimeter, etc. Shape analysis Circularity, aspect ratio, horizontal bounding rectangle aspect ratio, etc. Other parameters Particle area, average brightness, etc.	
Statistical analysis items	Average, standard deviation, CV, median (50 % value), mode value, user defined % value	
Display items	Particle image, histogram, scattergram, cumulative distribution, frequency/integration table, user defined area particle count	
Required sample volume	50 to 1000 µL	
Pump	Syringe pump with 0.1 mL/min flowrate	
Wetted part materials	Measurement unit:	PEEK resin, fluorine resin, quartz, or fluorine rubber
	Pump unit:	Fluorine resin or glass
Dimensions and weight	Measurement unit:	W 223 mm × D 465 mm × H 205 mm, 10 kg
	Pump unit:	W 97 mm × D 190 mm × H 150 mm, 3 kg

*1 Performance guarantee range of area circle equivalent diameter. Measured using a Shimadzu NIST traceable particle size standard sample.
*2 Measured using concentration standard samples specified by Shimadzu.

Evaluating the Thermal Stability of Proteins

Using a Differential Scanning Calorimeter to Measure the Thermal Stability of Proteins

click here

Operating Principle and Features

A differential scanning calorimeter (DSC) can measure the enthalpy changes in heat energy generated (endothermic or exothermic) as a sample is heated or cooled. Sample and reference solutions are placed in approx. 6 mm diameter cells, with the cells placed in thermally symmetric positions within the furnace, and then the furnace is heated or cooled at a constant rate. For example, when proteins are heated at a constant rate, denaturation can cause the three-dimensional structure to begin unfolding. DSC systems can measure the thermal changes that occur during that process as endothermic peaks (thermal denaturation temperatures). Due to its superior baseline stability, the DSC-60 Plus can easily measure the thermal changes of samples in solution.

Operating Procedure and Measurement Conditions

Samples were prepared by diluting lysozyme from chicken egg white with a phosphate buffer solution (pH 7.05) to the concentrations indicated in Table 1. Then, 20 µL of the sample was sealed with an aluminum hermetic cell. Using 20 µL of the phosphate buffer solution as a reference sample, the samples were heated from 35 to 105 °C at a rate of 5 °C per minute to measure the thermal denaturation temperatures.
To investigate the effect of protein pH, three 0.2 mol/L phosphate buffer solutions with pH 4.20, pH 7.05, and pH 9.10, were used as solvents for preparing and measuring the 10 % lysozyme solutions. The temperature was increased from 40 to 100 °C at a rate of 5 °C per minute.

Table 1 Samples Used

Sample	Concentration
Lysozyme in Fig. 1	2.5 % of protein
Lysozyme in Fig. 2	0.2 % of protein

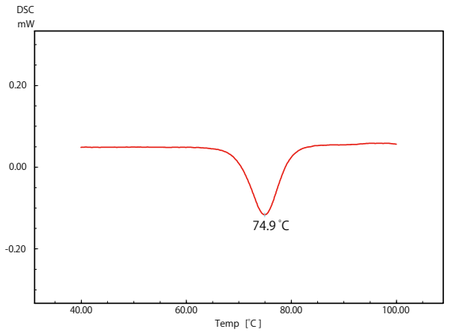


Fig. 1 Endothermic Peak of 2.5 % Lysozyme Solution

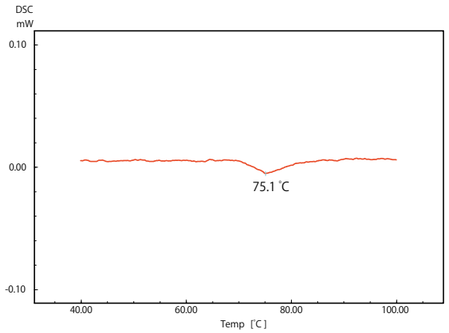


Fig. 2 Endothermic Peak of 0.2 % Lysozyme Solution

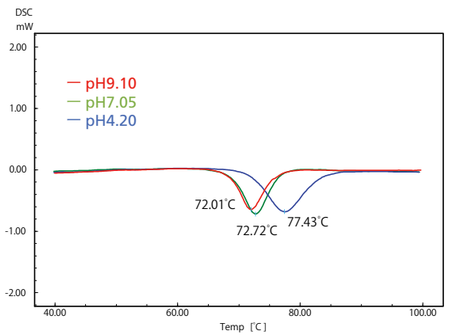


Fig. 3 Changes in Lysozyme Stability depending on pH

Results

With 0.2 % or 2.5 % lysozyme, endothermic peaks from thermal denaturation appear near 75 °C, which confirms that protein thermal denaturation temperatures can be measured in dilute 0.2 % solutions (Fig. 1 and 2).
It also shows that stability is highest for lysozyme with pH 4.20, which had the highest thermal denaturation temperature (Fig. 3).

Conclusion

DSC systems can easily measure the thermal denaturation temperatures of proteins and can be used for evaluating the thermal stability to provide an index for a variety of other evaluations, such as for evaluating the stability of modified proteins or considering different storage solvents.

Application Examples (Shimadzu Application News No.)

- Evaluating the stability of proteins
- Evaluating crystal polymorphism in pharmaceuticals (T152)

DSC-60 Plus

benefits

- The thermal stability of proteins can be easily evaluated.
- The stability due to pH or solvent differences can be evaluated.
- With the built-in liquid nitrogen cooling chamber, the system can be used to evaluate the protein effects of freezing.

Specifications

Instrument	DSC-60 Plus
Method	Heat flow
Measurement temperature range	-140 to 600 °C (when using liquid nitrogen with cooling chamber included standard)
Heat measurement range	±150 mW
Baseline noise	0.5 µW max. (RMS value for a blank held at 150 °C)
Atmosphere	Nitrogen, inert gas, or dry air gas flow
External dimensions	W 320 mm × D 500 mm × H 290 mm
Weight	28 kg
Power requirement	100 / 120 / 220 / 230 / 240 V AC ±10 %, 50/60 Hz, 800 VA
Optional	SSCP-1 sample sealer and crimp press Cell compatible with crimp attachment*1 Cell compatible with sealing attachment*2 Aluminum crimped cell*1 Aluminum sealed cell*2

*1 Used in Application News T152
*2 Used in Fig. 1 to 3 (p. 42)

Controlling Elemental Impurities

Analysis by ICP Mass Spectrometry Specified in the ICH Q3D Guideline for Elemental Impurities

click here

Operating Principle and Features

ICP-MS systems are generally considered to offer the highest sensitivity available for elemental analysis. These systems use an inductively coupled argon plasma (ICP) generated at about 10,000 °C to ionize elements in liquid samples and then analyze the ions by mass spectrometry with the ability to detect elements down to the ppt level.
Given that elemental impurities in pharmaceuticals must be controlled to very low concentrations, ICP-MS systems have been attracting attention because of their high sensitivity. They also have disadvantages, however, such as high argon gas consumption rates and high running costs. In contrast, ICPMS-2030 enables analysis at about one half the cost overall. This is because it features Shimadzu's unique mini-torch plasma system that successfully reduces argon gas consumption to 2/3 of conventional levels. Furthermore, relatively inexpensive 99.99 % pure industrial-grade argon gas can be used instead of 99.999 % or higher high-purity argon gas normally used for ICP-MS analysis.

Measurement Method and Conditions

The ICH Q3D Guideline for Elemental Impurities specifies Permitted Daily Exposure (PDE) for 24 elements for which toxicity is a concern. We have verified that ICPMS-2030 can adequately assess whether the guidelines are met. The mini-torch and low-cost industrial-grade argon gas were used.
One tablet (maximum daily dose of 0.20 g), 0.5 mL of hydrochloric acid, and 5 mL of nitric acid were placed in a quartz decomposition vessel and decomposed in a microwave sample pretreatment system. After decomposition, 0.1 mL of hydrochloric acid was added and the mixture was made up to 20 mL with pure water to prepare the measurement solution (100-fold dilution). Internal standard elements Sc, Ga, Y, and Te were also added during that process (to a 10 µg/L concentration of the measurement solution). Spike-and-recovery test solutions were prepared by adding measurement elements to the decomposed sample.
The calibration curve method with the internal standard method was used to quantitatively analyze and perform a spike-and-recovery test for the 24 elements subject to the ICH Q3D guideline according to the measurement conditions shown in Table 1.

Results

For many of the elements, the concentration measurement value was "N.D.", but concentration was nevertheless confirmed down to at least four digits below the permitted concentration. Recovery rates for each added element were also good, which clearly shows that measurements were performed correctly (Table 2).

Conclusion

With the ICPMS-2030 system, the 24 elements subject to the ICH Q3D Guideline can be analyzed quickly and accurately at half the running cost of conventional systems.

Table 1 Measurement Conditions

Instrument	ICPMS-2030
RF power	1.2 kW
Plasma gas flowrate	8.0 L/min
Auxiliary gas flowrate	1.1 L/min
Carrier gas flowrate	0.60 L/min
Sample injection	Nebulizer10
Chamber	Cyclone chamber (electronically cooled)
Plasma torch	Mini-torch
Collision gas	He

Table 2 Tablet Analysis Results

	Oral Preparation PDE	Permitted Concentration*1	Detection Limit (3 σ) Converted for Tablet Preparations*2	Measurement Value (in tablet)	Spiked Concentration (in tablet)	Spike and Recovery Rate
	µg	µg/g	µg/g	µg/g	µg/g	%
Ag	150	750	0.001	N. D.	0.1	107
As	15	75	0.002	N. D.	0.2	101
Au	100	500	0.001	N. D.	0.2	91
Ba	1400	7000	0.002	0.013	0.2	96
Cd	5	25	0.003	N. D.	0.2	96
Co	50	250	0.0006	N. D.	0.4	101
Cr	11000	55000	0.003	0.017	0.4	104
Cu	3000	15000	0.04	0.15	0.4	102
Hg	30	150	0.006	N. D.	0.2	100
Ir	100	500	0.0005	N. D.	0.2	98
Li	550	2750	0.01	N. D.	0.2	93
Mo	3000	15000	0.001	N. D.	0.2	107
Ni	200	1000	0.003	0.156	0.4	101
Os	100	500	0.007	N. D.	0.2	92
Pb	5	25	0.001	0.003	0.2	105
Pd	100	500	0.006	N. D.	0.2	104
Pt	100	500	0.003	N. D.	0.2	99
Rh	100	500	0.0008	0.003	0.2	101
Ru	100	500	0.002	N. D.	0.2	98
Sb	1200	6000	0.0009	0.007	0.2	98
Se	150	750	0.01	N. D.	0.2	98
Sn	6000	30000	0.002	N. D.	0.2	98
Tl	8	40	0.0005	N. D.	0.2	103
V	100	500	0.002	N. D.	0.4	100

*1 Permitted concentration	PDE level based on a daily intake of 0.2 g, which refers to a permitted concentration for oral preparations
*2 Detection limit converted for tablet preparations (3 σ)	Detection limit in measured solution (3 σ) × Dilution ratio (100)
N.D.	Not detected

Application Examples

- Measuring hazardous elements in public drinking water
- Analyzing hazardous elements and minerals in foods
- Analyzing components in blood
- Evaluating the safety of pharmaceuticals

ICPMS-2030

benefits

- Using Shimadzu's unique mini-torch plasma system, samples can be measured at half the running cost of previous systems.
- The collision cell offers high sensitivity and low interference to enable simultaneous measurement of all target elements evaluated.
- If the Development and Diagnosis Assistant functions, an industry first, are used, data can be analyzed in one tenth the time required previously. (Click here for the assistant functions.)

Specifications

	Instrument:	ICPMS-2030
Plasma ion source unit	Spray chamber:	Cyclone chamber (Thermoelectric cooling type)
	Peristaltic pump:	4-channel
	Plasma torch:	Mini-torch plasma system
	Nebulizer:	Coaxial
	Torch position adjustment:	X, Y, Z-axes automatic adjustment
RF power supply unit	Frequency:	27 MHz
	High-frequency output:	Max. 1.4 kW ± 0.3 %
Mass spectrometer unit	Mass analyzer:	Quadruple type mass spectrometer
	Mass range:	5 to 260
	Collision cell:	Octopole collision cell Helium gas 0 to 10 mL/min
	Detector:	Electron multiplier
	Exhaust system:	3-stage operation exhaust
	Dimensions:	W 870 mm × D 645 mm × H 587 mm (excluding protrusion)
	Weight:	145 kg

Pretreatment for Quantitative Analysis of Antibodies

Pretreatment Method Developed for Quantitative Analysis of Monoclonal Antibodies in Blood by LC-MS/MS

click here

Operating Principle and Features of Kit

The nSMOL Antibody BA kit can be used for all types of antibody drugs and eliminates the need for creating and cross-testing antibodies specifically for detection, which is typically required for detecting monoclonal antibodies. Advantages include a broader dynamic range and much higher selectivity than the ELISA method and the ability to analyze multiple components simultaneously. In terms of operation, performing the pretreatment on filter cups avoids tedious washing operations and samples can be collected easily by centrifuging (Fig. 1). After pretreatment, samples can be injected directly into the LC-MS system. On the plastic surface, the antibody collection resin is coated to ensure the Fab fragment side of antibodies is oriented toward the outside. By adding nanoparticles with trypsin solidified on the surface to the antibodies, the trypsin can access the antibodies efficiently for trypsin digestion (Fig. 2). The peptides derived from Fab fragments by trypsin digestion can be collected easily by centrifuging.

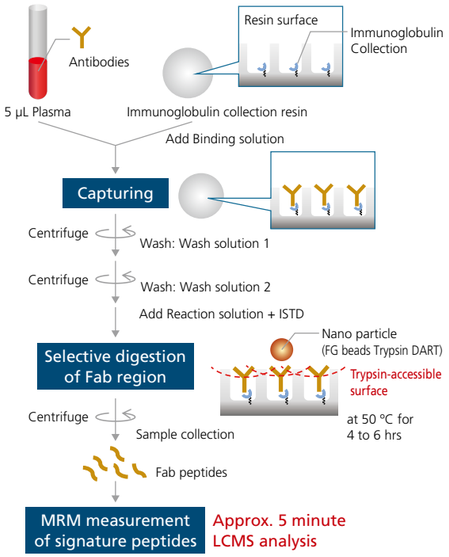


Fig. 1 Simple Workflow

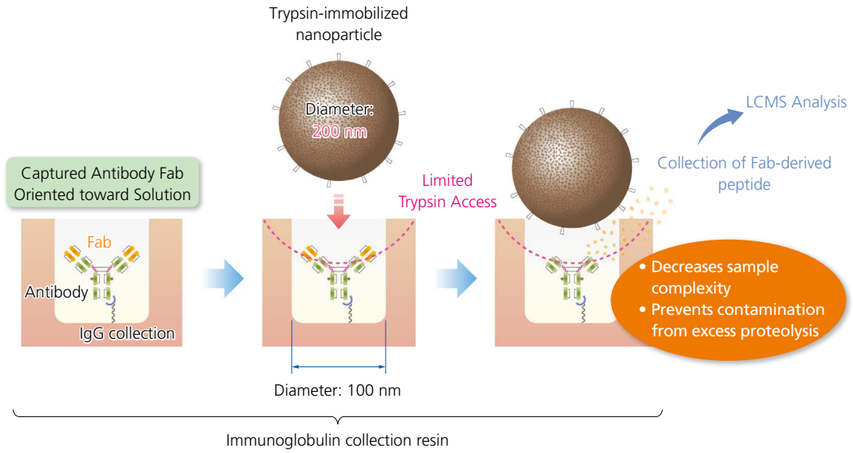


Fig. 2 Operating Principle of Kit

nSMOL Antibody BA Kit
Pretreatment Kit for LC/MS/MS Quantitative Analysis of Monoclonal Antibodies

benefits

- Selectively recovers Fab peptides and enables analysis without excessive peptides or trypsin.
- Offers general applicability for any type of antibody drug.
- No antibodies or ligands are needed for capturing, which streamlines method development and helps reduce costs.

Kit Contents

Reagent Name	Quantity	Capacity	Storage Temperature
Immunoglobulin collection resin	2	1.3 mL per vial	4 °C
Wash solution 1 (Binding solution)	1	80 mL	4 °C
Wash solution 2	1	80 mL	4 °C
Reaction solution	1	10 mL	4 °C
Enhanced reaction solution	1	Freeze-dried	4 °C
Reaction stop solution	1	1 mL	4 °C
FG beads Trypsin DART	1	1.1 mL	-20 °C*1

Note: The reagent kit is shipped refrigerated (2 to 8 °C).
*1 For long-term storage of 1 month or more, store at -80 °C.

Evaluating the Concentration of Antibody Drugs in Blood

LC/MS Bioanalysis of Antibody Drugs by nSMOL Fab-Specific Protein Analysis Method

—Example of Trastuzumab Analysis—

click here

Operating Principle and Features

Shimadzu's nSMOL method is a revolutionary LC/MS pretreatment method that enables Fab-specific protein decomposition of monoclonal antibodies. It enables the development of methods that do not depend on the type of antibody drug, which represents a paradigm shift for antibody drug analysis. It also satisfies criteria specified in the Guideline on Bioanalytical Method Validation in Pharmaceutical Development (Japanese Ministry of Health, Labour and Welfare). Shimadzu offers methods and protocols optimized for both. This method has been optimized for the Shimadzu LCMS-8050 and LCMS-8060 triple quadrupole mass spectrometers (referred to as "LCMS-8050" and "LCMS-8060 (NX)" below).

Measurement Method and Conditions

Human blood plasma spiked with trastuzumab was pretreated by the nSMOL method and then Fab-derived peptides were obtained. Then the content of trastuzumab in the blood plasma was quantitatively analyzed by LC-MS (Table 1). The results were fully validated in accordance with the Japanese Ministry of Health, Labour and Welfare Guideline on Bioanalytical Method Validation in Pharmaceutical Development.

Results

The peptide to be quantified (signature peptide) is selected from trypsin peptide fragments that include complementarity-determining regions (CDRs) that determine antibody specificity. However, even if a peptide contains CDRs, there is no guarantee its sequence is not identical to endogenous IgG. That requires confirming that they do not compete within the biological matrix used. However, given the operating principle of mass spectrometers, they can only obtain basic *m/z* and signal intensity information. Therefore, a data analysis method able to obtain high-quality and accurate analytical results by simultaneously using quantitative MRM settings for bioanalysis and using MRM monitoring for structural confirmation (Table 2 and Fig. 1) was used. That resulted in satisfying the Japanese Ministry of Health, Labour and Welfare guideline (Table 3) and obtaining a good calibration curve.

Conclusion

LC/MS quantitative analysis of antibody drugs in blood plasma (0.06 µg/mL lower limit of quantitation) can be performed using the same assay method for everything from preclinical testing to human clinical trials.

Application Examples

• Quantitating monoclonal antibodies in blood serum or blood plasma

Table 1 LC-MS Analytical Conditions

[LC] NexeraX2 system	
Column:	Shim-pack GISS C18 (50 mm × 2.1 mm I.D.)
Column oven:	50 °C
Solvent A:	0.1 % formic acid/water
Solvent B:	0.1 % formic acid/acetonitrile
Gradient: (Conc. B)	1 % (1.5 min) → 25 % (4.0 min) → → 95 % (5.0 min) → 1 % (6.0 min)
Flowrate:	0.4 mL/min
Injection:	10 µL
[MS] LCMS-8050, 8060	
Ionization:	ESI Positive
DL:	250 °C
Heat Block:	400 °C
Interface:	300 °C
Nebulizer gas:	3 L/min
Drying gas:	10 L/min
Heating gas:	10 L/min

Table 2 MRM Transitions of Quantified Peptides in Trastuzumab

Peptide	MRM transition	Purpose
$P_{14}R$	512.1 > 292.3 (b3+)	For quantitation (IS)
	512.1 > 389.3 (b4+)	For structural confirmation
	512.1 > 660.4 (b6+)	For structural confirmation
IYPTNGYTR	542.8 > 404.7 (y7++)	For quantitation
	542.8 > 808.4 (y7+)	For structural confirmation
	542.8 > 610.3 (y5+)	For structural confirmation

Note: Quantitation range in human blood plasma :0.0610 to 250 µg/mL
Averaged accuracy :100.7 %

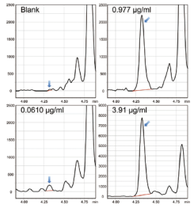


Fig. 1 MRM Chromatogram of IYPTNGYTR in Human Blood Plasma

Table 3 Full Validation Results

Precision and Accuracy			
Set Concentration (µg/mL)	Data Average (N = 15)	Accuracy (%)	CV (%)
2.93	2.58	88.1	8.2
200	211	106	5.6
Freeze-thaw Test			
Set Concentration (µg/mL)	Data Average (N = 5)	Accuracy (%)	Temp (°C)
2.93	2.87	98.1	-20
200	199	99.7	-20
Long-term Stability Test			
Set Concentration (µg/mL)	Data Average (N = 5)	Accuracy (%)	Temp (°C)
2.93	3.03	104	-20
200	209	101	-20
Processed Sample Stability for 48 Hours			
Set Concentration (µg/mL)	Data Average (N = 5)	Accuracy (%)	Temp (°C)
2.93	3.67	91.2	5
200	211	106	5

index

LCMS-8050 / 8060 / 8060NX

benefits

- UF Technologies provide both maximum sensitivity and maximum speed.
- Due to an ultra-fast 5 msec polarity switching speed, positive and negative ions can be measured simultaneously.
- "Easy Maintenance" features lead to greater uptime.

Specifications

Model		LCMS-8050	LCMS-8060	LCMS-8060 NX
Mass range		*m/z* 2 to 2000	*m/z* 2 to 2000	*m/z* 2 to 2000
Sensitivity	ESI positive	1 pg reserpine, S/N > 500,000:1 (RMS)	1 pg reserpine, S/N > 1,500,000:1 (RMS)	1 pg reserpine, S/N > 1,500,000:1 (RMS)
	ESI negative	1 pg chloramphenicol, S/N > 500,000:1 (RMS)	1 pg chloramphenicol, S/N > 1,500,000:1 (RMS)	1 pg chloramphenicol, S/N > 1,500,000:1 (RMS)
Resolution		R < 0.7 u (FWHM) and adjustable to 0.5 u	R < 0.7 u (FWHM) and adjustable to 0.5 u	R < 0.7 u (FWHM) and adjustable to 0.5 u
Mass stability		0.05 u/24 hr	0.05 u/24 hr	0.05 u/24 hr
Mass accuracy		0.1 u	0.1 u	0.1 u
Scan speed		Max. 30,000 u/sec	Max. 30,000 u/sec	Max. 30,000 u/sec
Polarity switching time		5 msec	5 msec	5 msec
Interface		Standard: ESI	Standard: ESI	Standard: IonFocus (ESI, DUIS)
		Optional: Micro-ESI, APCI, DUIS	Optional: Micro-ESI, APCI, DUIS	Optional: Micro-ESI, APCI

index

Evaluating the Concentration of Antibody Drugs in Blood

High-Sensitivity LC/MS Bioanalysis of Trastuzumab by nSMOL

click here

Operating Principle and Features

High-performance liquid chromatograph mass spectrometer (LC-MS) systems enable higher performance analysis by decreasing the flowrate in the LC unit and improving the ionization and ion uptake efficiency in the MS unit. The Shimadzu Nexera Mikros is a micro LC-MS system that reduces the LC flowrate to a micro level (approx. 1 to 10 µL/min). That results in between several times to several tens of times higher sensitivity than the previous semi-micro LC-MS system, while maintaining the same robustness and throughput.

Measurement Method and Conditions

The ELISA ligand-binding assay method was the primary method used to determine the concentration of antibody drugs in the blood. This example describes a quantitative method that is based on using a high-sensitivity LC-MS system. Human blood plasma spiked with a trastuzumab standard and blank blood plasma were analyzed as samples. For all the antibody drugs, Fab-derived peptides were obtained using the nSMOL method, which allowed using the same protocol for all samples. These were analyzed to quantify the concentration of trastuzumab in the blood plasma based on the analytical conditions indicated in Tables 1 to 3.

Table 1 LC Analytical Conditions

[Analytical]:	
Column:	Shim-Pack MC C18 (50 mm × 0.175 mm I. D., X µm)
Mobile phase A:	0.1 % Formic acid in water
Mobile phase B:	0.1 % Formic acid in Acetonitrile
Gradient: (B. Conc.)	5 % (0 – 0.5 min) → → 25 % (0.5 – 4.5 min) → → 95 % (4.51 – 5.0 min) → → 5 % (5.01 – 11.0 min)
Flowrate:	4.0 µL/min
Column Temp.:	50 °C
[Trap]	
Trap column:	L-column 2 ODS Micro (5 mm × 0.3 mm I.D., X µm)
Mobile phase A:	0.05 % Trifluoroacetic acid in water
Mobile phase B:	0.1 % Formic acid in Acetonitrile
Column Temp.:	50 °C
Injection Volume:	10 µL

Table 2 MS Analytical Conditions

Ionization:	ESI Positive
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
ESI Temp.:	100 °C
Nebulizer Gas:	2 L/min
Drying Gas:	OFF
Heating Gas:	3 L/min

Conclusion

Using the nSMOL method in combination with the Nexera Mikros system enables high-sensitivity quantitation of antibody drugs in blood without sacrificing throughput.

Table 3 MRM Transitions of Quantified Peptides in Trastuzumab

Peptide	MRM transition	Purpose
$P_{14}R$	512.1 > 292.3 (b3+)	For quantitation (IS)
	512.1 > 389.3 (b4+)	For structural confirmation
	512.1 > 660.4 (b6+)	For structural confirmation
IYPTNGYTR	542.8 > 404.7 (y7++)	For quantitation
	542.8 > 808.4 (y7+)	For structural confirmation
	542.8 > 610.3 (y5+)	For structural confirmation

Note: The peptide (signature peptide) was selected from trypsin peptide fragments that include complementarity-determining regions (CDRs) that determine antibody specificity.

Results

Measured concentrations of trastuzumab in blood plasma correlated closely with concentration settings, with an R^2 value of 0.99 or higher and excellent accuracy and precision. Furthermore, the Nexera Mikros system was also used to confirm that the LLOQ value for trastuzumab in blood plasma is 0.00763 µg/mL.
A comparison of MRM chromatograms from the Nexera Mikros system (Fig. 1) and a typical semi-micro LC-MS system (Fig. 2) shows that the Nexera Mikros is able to detect with ample sensitivity concentrations that the semi-micro LC-MS system was not able to detect.

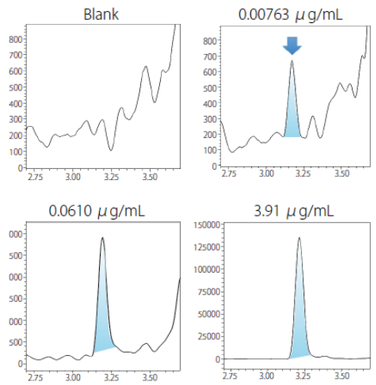


Fig. 1 MRM Chromatograms (Nexera Mikros)

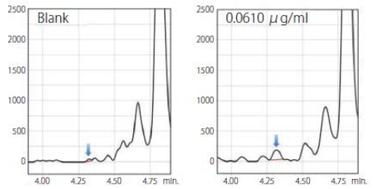


Fig. 2 MRM Chromatograms (Semi-Micro LC-MS)

Application Examples

- Analyzing lipid mediators with high sensitivity

Nexera Mikros

benefits

- Micro-flowrate LC-MS system that offers both high sensitivity and high throughput.
- Easy one-step attachment of analytical columns and connection to the LC-MS ionization interface.

Direct injection system: Small injection volumes make it especially well-suited for analyzing desalted or otherwise pretreated samples.

Trap and elute system: System for increasing sensitivity of semi-micro systems without changing the injection volume or other parameters

Ionization promotion system: System for increasing negative mode sensitivity by adding an LC-20AD nano unit to each of the two systems above

Specifications

Solvent delivery unit	LC-Mikros	Flowrate range:	1 to 500 µL/min
System pressure capacity	80 MPa		
Autosampler	SIL-40C XR	Injection volume range:	0.1 to 50 µL
Ionization unit	Micro-ESI 8060 or Micro-ESI 9030		
Column oven	CTO-Mikros (UF-Link enables connecting/disconnecting columns easily with zero dead volume.)		

Comprehensive Metabolite Analysis

Comprehensive Analysis of All Metabolites Using GC/MS and LC/MS for Researching Intestinal Bacteria

click here

Operating Principle and Features

Metabolomic analysis using a mass spectrometer generally involves using a gas chromatograph mass spectrometer (GC-MS) or high-performance liquid chromatograph mass spectrometer (LC-MS) to comprehensively analyze all the metabolites (metabolome) contained in a sample. That requires selectively using GC/MS or LC/MS based on the target components being analyzed or the given purpose of analysis, as illustrated in Fig. 1. Using a GC/MS to analyze hydrophilic metabolites such as amino acids, organic acids, or sugars requires a derivatization process, but it offers superior robustness and can comprehensively analyze hundreds of components in a single analysis. In contrast, an LC/MS can efficiently analyze specific metabolites (up to 100 components) without derivatization, making it well-suited for routine analysis of specific components.

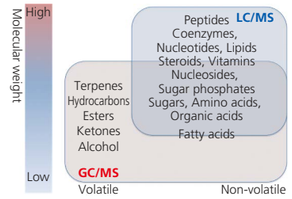


Fig. 1 Target Components for GC/MS and LC/MS Analysis

Measurement Method and Conditions

Fresh fecal samples were collected from male C57BL/6J mice raised in a normal environment. 450 µL of a physiological phosphate buffer solution was added to 50 mg of the fecal samples and then stirred. Then the supernatant was ultrafiltered by centrifugal separation to extract the metabolites. To analyze the primary metabolites by GC-MS/MS, the filtrate was derivatized to prepare the samples for GC-MS/MS. Meanwhile, to analyze the primary metabolites by LC-MS/MS, the filtrate was diluted by ten times with ultrapure water in preparation for LC-MS/MS.
For the GC/MS/MS analysis, 475 components were analyzed simultaneously using an MRM method from the Smart Metabolites Database, which includes MRM information for 475 components, mainly for metabolites included in biological samples. For the LC/MS/MS analysis, a method of ion pair LC/MS/MS and a method of ion pair-free LC/MS/MS were used for analysis in LCMS-8040 and LCMS-8050 systems. The method of ion pair LC/MS/MS is intended for simultaneous analysis of 55 metabolite components important for metabolomic analysis in the life sciences, such as for analyzing the glycolytic system, TCA cycle, pentose phosphate pathway, or amino acids/nucleotides, whereas the method of ion pair-free LC/MS/MS is intended for simultaneous analysis of 97 organic acid and other metabolite components that cannot be analyzed using the method of ion pair LC/MS/MS. Both methods are included in the LC/MS/MS Method Package for Primary Metabolites Ver. 2.

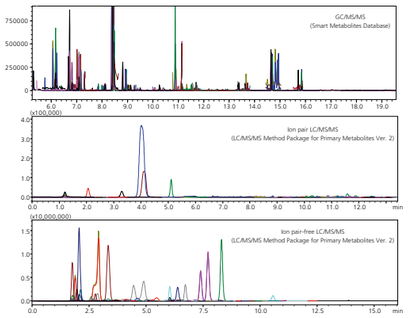


Fig. 2 MRM Chromatograms of Mouse Fecal Extract

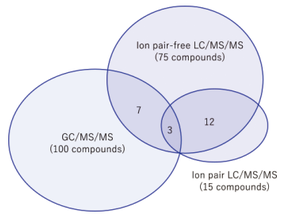


Fig. 3 Number of Metabolites Detected from Mouse Fecal Extract

Results

The GC/MS/MS analysis detected 100 components, mostly short-chain fatty acids and organic acids. It even detected 17 sugar components that are difficult to analyze by LC or LC/MS/MS (Fig. 2). The ion pair method detected 17 components, including mainly amino acids. The ion pair-free method detected 75 components, including amino acids, nucleotides, nucleosides, and organic acids involved in the TCA cycle (Fig. 2). Therefore, it is extremely useful to use both GC/MS/MS and LC/MS/MS for comprehensively analyzing metabolites in fecal samples.

Conclusion

Because GC/MS/MS and LC/MS/MS methods target different components, comprehensive analysis of metabolites is enabled by using both methods. Furthermore, by using the MRM database for GC/MS/MS analysis and method packages for LC/MS/MS analysis, comprehensive analysis can be easily performed by operators who are not very familiar with this analysis. The large amounts of data generated can be interpreted easily by using Shimadzu's Multi-Omic Data Analysis package to visualize the data. These sample and data analysis methods should be extremely useful not only for researching intestinal flora, but also for metabolomic analysis in a wide variety of other pharmacokinetic applications.

Application Examples

- Simultaneous analysis of metabolites (metabolomics)

This article was prepared with generous cooperation from Takanari Hattori[*1,*2], Akihiko Kunisawa[*1,*2], Shuichi Kawano[*1], Shinichi Kono[*1,*2], Yoshihiro Hayakawa[*1], Junko Iida[*1,*2], Eiichiro Fukusaki[*2,*3], Mitsuharu Matsumoto[*4].
*1 Analytical & Measuring Instruments Division, Shimadzu Corporation
*2 Osaka University and Shimadzu Analytical Innovation Research Laboratory
*3 Graduate School of Engineering, Osaka University
*4 Research Laboratories, Kyodo Milk Industry Co., Ltd.

GCMS-TQ8040 NX
LCMS-8040 / 8050

benefits

- The world's largest metabolite database includes preregistered optimized methods.
- MRM measurements can detect components not detectable by scan or SIM modes.
- High-speed MRM analysis enables simultaneous analysis of multiple components to achieve comprehensive high-sensitivity analysis.

Specifications

Instrument	GCMS-TQ8040 NX
GC unit	Oven temperature: Room temperature + 2 to 450 °C Carrier gas control: Constant liner velocity, constant pressure, or constant flowrate Flow controller pressure: Max. 970 kPa
MS unit	Ionization: EI (standard), CI, NCI (optional) Mass range: *m/z* 10 to 1090 MRM max. speed: >800 MRM/sec Measurement modes: Scan mode, SIM mode, MRM mode, product ion scan mode, precursor ion scan mode, and neutral loss scan mode, or simultaneous analysis with any combination thereof. Weight: 110 kg for GC-MS main units and 10 kg for auxiliary pump

Biomarker Discovery by Volatile Gas Analysis

Analysis of Volatile Gases Generated by Intestinal Microorganisms

click here

Operating Principle and Features

Microorganisms (flora) in intestines generate a wide variety of volatile substances. Comprehensive analysis of such flora is used for biomarker discovery and other research. Volatile substances can be comprehensively analyzed using a gas chromatograph mass spectrometer in combination with a headspace sampler unit (HS-20 + GCMS-QP2020 NX).
Volatile sulfur compounds can be analyzed with high sensitivity by using the headspace sampler in combination with an SCD detector that detects only sulfur components with high sensitivity (HS-20 + Nexis GC-2030 + SCD-2030).

Measurement Method and Conditions

Fresh fecal samples from both germ-free and flora-intact mice were placed directly into headspace vials. The vials were filled with anaerobic gas, sealed, and left to cultivate for 24 hours. Then the vials were placed in the headspace sampler and the evolved gases were analyzed by GC/MS and GC-SCD. (For detailed analytical conditions, refer to the site linked to the title.)

Results

The total ion chromatogram (TIC) from a comprehensive analysis of volatile substances (Fig. 1) and the chromatogram from analyzing sulfur-based volatile substances (Fig. 2) both confirmed that a larger number and quantity of volatile substances were detected from the flora-intact mouse than the germ-free mouse. They also confirmed that GC-SCD analysis can detect and identify sulfur compounds, which can have low peak intensity or overlap with other peaks in GC/MS results.
GC/MS data for 121 types of compounds acquired from two samples taken from each of six mice with intact flora was analyzed by principal component analysis using SIMCA15 multivariate analysis software. In the score plot obtained from the above measurements (Fig. 3), the smallest clusters were formed from samples taken from the same individual. The individual-specific clusters clearly show that the system can detect differences between individuals.

Conclusion

This example confirmed that volatile gases generated from intestinal flora can be analyzed without pretreatment using GC/MS and GC-SCD. GC/MS enables comprehensive analysis, whereas GC-SCD enables analysis of low-concentration sulfur components that are difficult to detect by GC/MS. Using the methods in combination can be useful for biomarker discovery and other pharmacokinetic applications.

Application Examples

- Comprehensive analysis of volatile components
- High sensitivity analysis of volatile sulfur compounds
- Discovery of new biomarkers

GC/MS (Comprehensive Analysis of Volatile Substances)

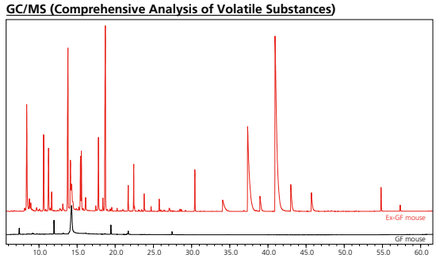


Fig. 1 GC/MS Total Ion Chromatogram

GC-SCD (Selective Analysis of Sulfur-Based Volatile Substances)

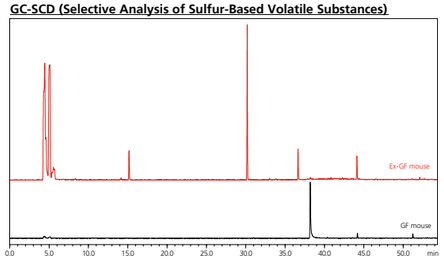


Fig. 2 GC-SCD Chromatogram

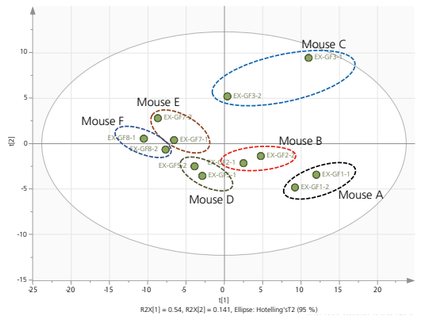


Fig. 3 Results (Score Plot) from Multivariate Analysis of Flora-Intact Mouse Analysis Results

We would like to acknowledge Principal Investigator Dr. Mitsuharu Matsumoto at Kyodo Milk Industry Co., Ltd. for providing samples and other help with conducting the above measurements.

index

HS-20 Trap / GCMS-QP2020 NX
HS-20 / Nexis GC-2030 / SCD-2030

benefits

- The HS-20 headspace sampler enables high-sensitivity analysis of volatile gases without pretreatment.
- Using the HS-20 trap mode enables even higher sensitivity.
- The GC-SCD system enables selectively analyzing sulfur components with high sensitivity.

Specifications

Instrument	HS-20 Trap / GCMS-QP2020 NX
HS unit	Sample injection methods: Sample loop or adsorbent trap Samples: 90 Oven temperature: Room temperature + 10 to 300 °C (1 °C steps) Weight: 40 kg (HS-20Trap)
GC unit	Oven temperature: Room temperature + 2 to 450 °C Carrier gas control: Constant liner velocity, constant pressure, or constant flowrate Flow controller pressure: Max. 970 kPa
MS unit	Ionization: EI (standard), CI, NCI (optional) Mass range: *m/z* 1.5 to 1090 Measurement modes: Scan, SIM, and scan/SIM simultaneous measurement High-speed scan rate: 20,000 u/sec Weight: 85 kg for GC-MS main units and 10 kg for auxiliary pump

Specifications

Instrument	HS-20/Nexis GC-2030 (SCD-2030 detector)
HS unit	Sample injection method: Sample loop method Samples: 90 Oven temperature: Room temperature + 10 to 300 °C (1 °C steps) Weight: 33 kg (HS-20)
GC unit	Oven temperature: Room temperature + 2 to 450 °C Sample injection unit temperature: Max. 450 °C Carrier gas control: Constant liner velocity, constant pressure, or constant flowrate Flow controller pressure: Max. 970 kPa Weight: 43.5 kg (SPL/FID model)
SCD unit	Minimum detection quantity: <0.3 pgS/s (dibenzothiophene) Dynamic range: $>10^4$ Selectivity: $>10^7$ (S/C) Weight: 27 kg

index

Biomarker Discovery

Profiling Cancer Cells Using a Benchtop MALDI-TOF MS System

click here

Operating Principle and Features

Compared to quadrupole or magnetic sector mass spectrometers, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometers offer the advantage of both a broad mass measurement range and fast measurement speed. They are especially well-suited for measuring nucleic acids, proteins, and molecules. Though a benchtop system with a compact size, the MALDI-8020 offers world-class resolution and sensitivity levels. In addition, a shorter vacuum evacuation time is achieved by increasing the laser speed and modifying the exhaust system, and a significantly shorter measurement time is achieved by increasing stage speed.

Measurement Method

Cancer cells can metastasize throughout the body by means of extracellular vesicles. Therefore, a MALDI-TOF MS system was used to profile differences in the expression level of proteins derived from extracellular vesicles from regular lymph node cells metastasized from colon cancer versus from lymph node cells with elevated chemotherapy resistance.
Protein was collected from the extracellular vesicles obtained from cultivated cells and then the MALDI-8020 system was used to obtain a mass spectrum. Alpha-cyano-4-hydroxycinnamic acid (CHCA) was used as a matrix. eMSTAT Solution was used to analyze the resulting mass spectrum by multivariate analysis (Fig. 1).

Results

Components derived from the protein were detected in the *m/z* 2,000 to 25,000 range of the extracellular vesicle mass spectrum. Multivariate analysis score plot results discriminated between groups with resistance versus groups with sensitivity to fluorouracil. From the peak matrix, peaks that characterized chemotherapy resistance were detected in the *m/z* 2,000 to 7,000 range (Fig. 2).

Fig. 1 Workflow for Extracellular Vesicle Analysis

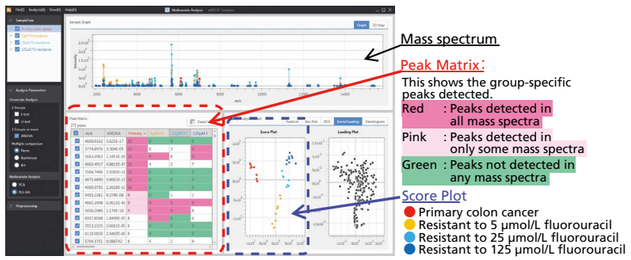


Fig. 2 PLS-DA Analysis Results for Four Extracellular Vesicle Groups

Conclusion

Using the MALDI-8020 system in combination with statistical analysis software shows its potential for use in biomarker discovery research. This type of protein profiling method can be expected to be useful for less invasive cancer diagnosis or for monitoring chemotherapy.

Application Examples (Shimadzu Application News No.)

- Profiling protein expression in tissue
- Analyzing glycans and glycopeptides (B113)
- Analyzing the primary structures of protein (B105)

The extracellular vesicles and cell extracts from primary colon cancer and metastasized lymph node subclones with resistance to 5, 25, and 125 µM concentrations of 5-fluorouracil (FU) were provided by Dr. Gerald Stubiger of the Medical University of Vienna.

MALDI-8020

benefits

- Enables rapid and highly sensitive benchtop profiling.
- Easy maintenance and low running costs
- Can search a wide range of molecular weights for nucleic acids, proteins, and molecules.

Specifications

Instrument		MALDI-8020
Mass range		*m/z* 1 to 500,000
Mass resolution		>5,000 FWHM
Sensitivity		>250 amol
Mass accuracy		<20 ppm with internal calibration, <150 ppm with external calibration
Acceleration voltage		15 kV
Laser		Solid-state laser wavelength: 355 nm Repetition frequency: 50, 100, or 200 Hz (variable)
Flight distance		850 mm
Detector		Electron Multiplier
Ion source cleaning		Includes automatic cleaning functionality (depending on built-in solid-state laser)
Sample plate		Disposable FlexiMass-DS and stainless steel FlexiMass-SR
Operating noise		<55 dB
Main unit power supply		Single-phase 100 V AC, 50/60 Hz, 1 kVA
Dimensions		W 600 mm × D 745 mm × H 1,055 mm (excluding protrusions)
Weight		86 kg
Operating environment		Temperature: 18 to 28 °C Humidity: Max. 70 % (with no condensation)
Data analysis software		eMSTAT Solution
Data analysis functionality	Univariate analysis	t-Test, Mann-Whitney U-Test, ANOVA (analysis of variance)
	Multivariate analysis	PCA (principal component analysis), PLS-DA
	Discriminant analysis	Support Vector Machine (SVM), Random Forest
	Other	Dynamic grouping
Display functionality	Multivariate analysis	Peak Matrix, Box Plot, ROC, AUC, Score/Loading Plot, Dendrogram
	Discriminant analysis	Discriminant analysis results (Group, Score) superimpose points for unknown samples on a score plot
Input/output data	Input	Peak list (ASCII, JCAMP, or mzML format)
	Output	Peak list (txt format), data analysis results (xlsm format), graph screenshot

TORAST-H Series

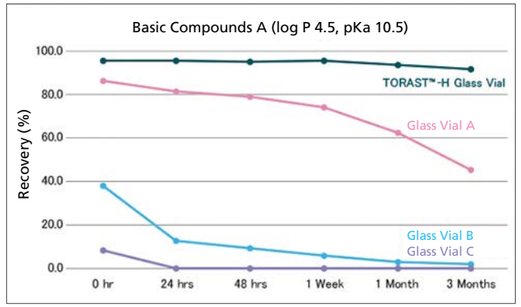
Shimadzu original low adsorption glass vial
TORAST-H Glass Vial

benefits

- Low adsorption glass vial suitable for long-term storage.
- Minimized adsorption of bases, acids and neutrals
- Superior quality control

Adsorption Test for Long-term Storage

When a sample is stored in a general vial for a long time, the sample may adsorb into the surface of the vial, causing the reproducibility to be poor. The TORAST-H Glass Vial contains low adsorption characteristics that makes it excellent for long-term sample storage.



	0 hr	24 hrs	48 hrs	1 Week	1 Month	3 Months
TORAST™-H Glass Vial	96.0 %	95.7 %	95.4 %	95.6 %	93.7 %	91.9 %
Glass Vial A	86.6 %	81.4 %	79.2 %	74.4 %	62.4 %	45.5 %
Glass Vial B	38.1 %	13.0 %	9.6 %	5.9 %	3.1 %	2.2 %
Glass Vial C	8.5 %	N.D.	N.D.	N.D.	N.D.	N.D.

Note: The area at time 0 of the PP vial (Controls) was set at 100 %.
After 3 months, the recovery rate of PP vials was 89 %, which was lower than that of TORAST-H Glass Vial.

Product	Details	Cap	Slit	Volume	Qty.	P/N
TORAST-H Glass Vial (Includes the quality certificate and PTFE/Silicone septum)	Clear glass with a label (wide diameter 9-425)	Screw, Black	No	1.5 mL	100	370-04300-01
			Yes			370-04300-02
	Amber glass with a label (wide diameter 9-425)		No			370-04300-03
			Yes			370-04300-04
	Clear glass with a label (wide diameter 9-425)		No	150 µL		370-04301-01
			Yes			370-04301-02
	Amber glass with a label (wide diameter 9-425)		No			370-04301-03
			Yes			370-04301-04
Screw Cap for TORAST-H Glass Vial	PTFE/Silicone septum (wide diameter 9-425)		No	—		370-04310-01
			Yes			370-04310-02

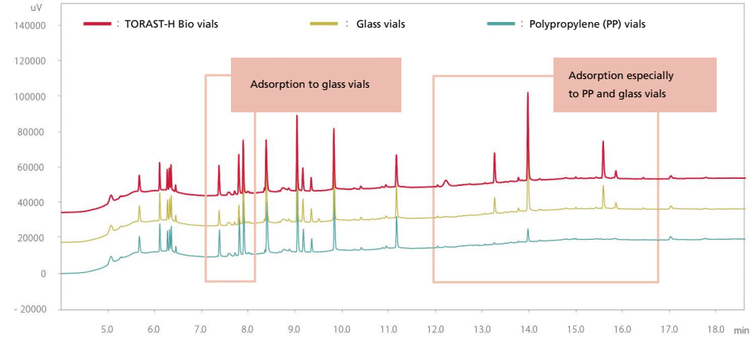
Shimadzu original low adsorption polypropylene vial
TORAST-H Bio Vial

benefits

- Extremely low adsorption of peptides
- Extremely low adsorption of basic compounds
- User-friendly design

Adsorption Test Using Trypsin Digestion Products of Myoglobin (approx. 1.9 pmol/mL)

The results confirmed the phenomenon that highly polar peptides with retention times detected between approx. 7 and 8 minutes mostly adsorb to glass vials, whereas highly hydrophobic peptides with retention times detected between approx. 12 and 16 minutes mostly adsorb to polypropylene (PP) vials.



Exterior Design that Enables Using Vials Directly for Flash Centrifugation

Product	Details	Slit	Volume	Qty.	P/N
TORAST-H Bio Vial	PP vial	Yes	300 µL	100	370-04350-00

If using a Shimadzu i-Series LC system, use the special vial detection plate (P/N: 228-51891-03) shown to the right.

Spectrofluorophotometer

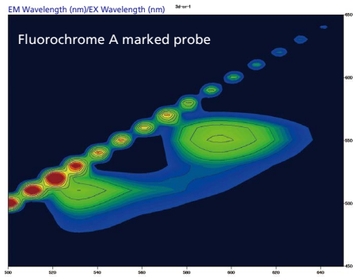
RF-6000

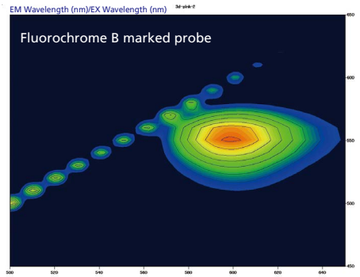
benefits

- Highest S/N Ratio in its class: 1,000 or more (RMS) /350 or more (P–P)
- High-speed scanning of 60,000 nm/min minimizes scan time.
- 2,000 hour long-life Xenon lamp
- Spectrum-Corrected Excitation and Emission spectra can be scanned.

Fluorescent Dyes for DNA Detection

Specified complementary DNA can be detected by using a DNA probe which is marked by fluorochrome. These probes become luminescent when bonded to DNA.
The following shows the results of a 3D measurement of DNA marked by two different kinds of DNA probes. Unique fluorescent peaks and profiles (3D Emission-Excitation matrix, as shown below) can be quickly measured using the high-speed scanning function.





RF-6000

Analytical Balances

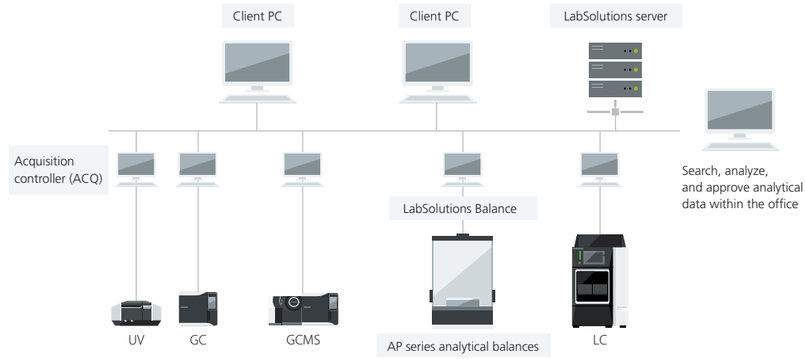
AP Series

Minimum display value:
0.1 mg or 0.01 mg

benefits

- Supports LabSolutions Balance chromatography data integrity.
- Increases productivity with the fastest response performance in its class.
- Using the internal windbreak plate in combination with a STABLO-AP ionizer ensures reliable results.

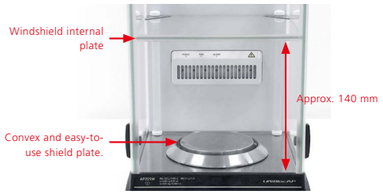
Integrated Management of Analytical Data via a Network System Using LabSolutions



Using the Internal Windbreak Plate in Combination with a STABLO-AP Ionizer Ensures Reliable Results

Internal Windbreak Plate

The internal windbreak plate suppresses the influence of convection and airflow within the weighing chamber, improving the stability and response of measurement values.



STABLO-AP Ionizer STABLO AP

Sweeps away static electricity from containers and samples!
It can be used as an external stand configuration or installed inside the balance unit.

External stand configuration

Internal installation configuration

AP Series

References:

Culture

p. 12_Monitoring of Metal Elements in Cell Culture Supernatant using Atomic Absorption Spectrometry

1) Inn H Yuk et al., Biotechnology Progress, 30, 429-442 (2014)
2) Prabhu et al., Applied Microbiology and Biotechnology, 102, 5989-5999 (2018)
3) Application News A634 “Direct Analysis of Metallic Elements in Cell Culture Medium by Atomic Absorption Spectrophotometry (AAS)”

p. 14_Simultaneous Analysis of Components in CHO Cell Culture Supernatant for Optimization of the Culture Process

1) Zhiyuan Sun et al., Biologicals, 61, 144-51 (2019)

Quality Control

p. 26_Analysis of Protein Secondary Structures—Analysis on Changes of Secondary Structures in Egg White Proteins Caused by Thermal Denaturation—

1) JENNIFER KOVACS-NOLAN, J. Agric. Food Chem., 53, 8421-8431 (2005)
2) Yoshinori Mine et. al., J. Agric. Food Chem., 38 (12), 2122–2125 (1990)
3) Jilie KONG, Shaoning YU., Acta Biochim. Biophys. Sin., 39(8), 549–559 (2007)
4) A. Kato and T.Takagi, J. Agric. FoodChem., 36, 1156-1159 (1988)

p. 30_*N*-Linked Glycan Analysis Using MALDImini-1—Structural Analysis and Identification of Sialyl Linkage Isomers

1) Nishikaze T, et al., Anal Chem, 89, 2353-2360 (2017)
2) Hanamatsu H, et al., Anal Chem, 90(22), 13193-13199 (2018)

p. 40_Characterization of Insoluble Subvisible Particles in Biopharmaceuticals Using the Flow Imaging Method

1) Susumu Uchiyama, Yakugaku Zasshi (Journal of the Pharmaceutical Society of Japan), 138, 1503-1507(2018)
2) Kiyoshi M et al., Journal of Pharmaceutical Sciences, 108, 832-841 (2019)

p. 44_Analysis by ICP Mass Spectrometry Specified in the ICH Q3D Guideline for Elemental Impurities

1) Guideline for Elemental Impurities in Drug Products (PFSB/ELD Notification No. 4, September 30, 2015)
2) GUIDELINE FOR ELEMENTAL IMPURITIES Q3D(R1)
3) Supplement II to the Japanese Pharmacopoeia 17th Edition (June 28, 2019, Ministry of Health, Labour and Welfare Notification No. 49)

Note: Currently, biopharmaceuticals are not subject to ICH Q3D.

Pharmacokinetics

p. 48_LC/MS Bioanalysis of Antibody Drugs by nSMOL Fab-Specific Protein Analysis Method—Example of Trastuzumab Analysis—

1) Iwamoto N et. al., Analyst, DOI: 10.1039/c3an02104a
2) Iwamoto N et. al., Anal Methods, DOI:10.1039/c5ay01588j

p. 50_High-Sensitivity LC/MS Bioanalysis of Trastuzumab by nSMOL

1) Iwamoto N et.al., Analyst, DOI: 10.1039/c3an02104a
2) Iwamoto N et al., Anal Methods, DOI: 10.1039/c5ay01588j
3) Application News C145A

p. 52_Comprehensive Analysis of All Metabolites Using GC/MS and LC/MS for Researching Intestinal Bacteria

1) M. Matsumoto, et al., Scientific Reports, 2, 223 (2012).

p. 56_Profiling Cancer Cells Using a Benchtop MALDI-TOF MS System

1) Stübiger, G. et al., Analytical Chemistry, 90, 13178-13182 (2018)

Shimadzu Corporation
www.shimadzu.com/an/

© Shimadzu Corporation, 2021 / First Edition: August 2021, 3655-05104-PDFIT, C10G-E089