C10G-E097

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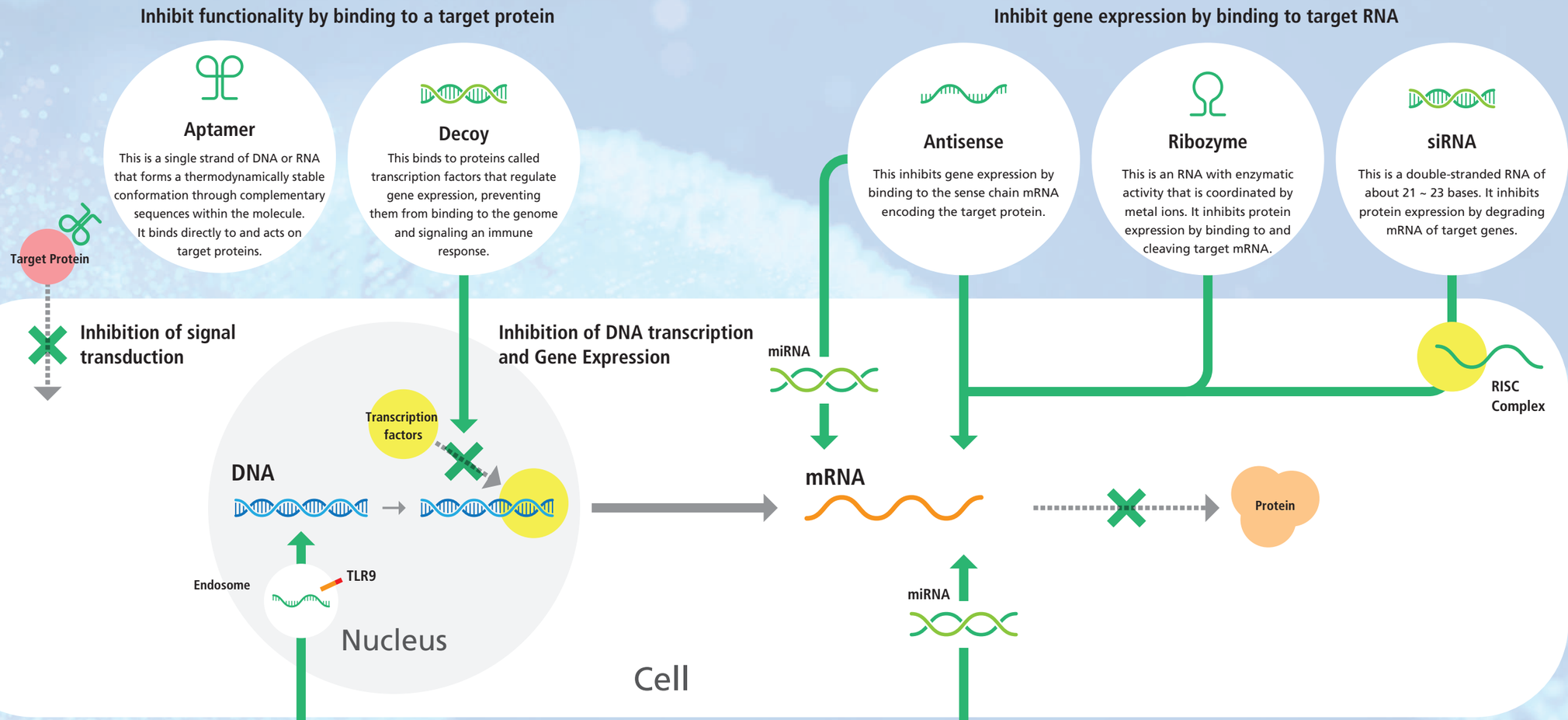
Oligonucleotide Therapeutics Solution Guide

Types and Characteristics of Oligonucleotide Therapeutics

Oligonucleotide therapeutics are nucleic acid polymers generally comprised of a few to several dozen bases (including modified bases) linked together. They are produced by chemical synthesis and act directly on organisms without being translated into proteins. Oligonucleotide therapeutics are characterized by the ability to target specific diseases. Another advantage is that it takes less time than conventional methods to find new therapeutic candidates because oligonucleotides are easy to design and synthesize.

However, it is a practical problem that oligonucleotide therapeutics are degraded and excreted rapidly after administration by exonucleases and endonucleases that are abundant in blood and cells. This problem is being addressed by the introduction of modified oligonucleotides to improve chemical stability in vivo and by the development of lesion-targeted DDS (drug delivery systems) technologies.



Note: Not all oligonucleotides therapeutics are applicable.

Workflow of Oligonucleotide Therapeutics

The workflow for research and development of oligonucleotide therapeutics is as follows. Various solutions for purification, characterization and quality control which are indispensable for the development of oligonucleotide therapeutics are introduced in this guide.

Target selection
Modification

Oligomer synthesis
Excision
Unprotected

Purification

Characteristic analysis
Quality Control

Pharmacokinetics
DDS

Separation of impurities

→Oligonucleotide therapeutics are mainly manufactured by chemical synthesis. Separation and purification from impurities such as base defect and residual protecting group formed in the synthesis process are large problems.

P. 6 – 9

Tm measurement and thermodynamic parameter analysis

→The melting temperature (Tm) of an oligonucleotide is the temperature at which 50% of double-stranded DNA or RNA separates into single strands. The higher the Tm, the more stable the double-stranded DNA or RNA. It is one of the quality control test items.

P. 10 – 13

Sequence confirmation

→The principle of quality control is "efficacy and safety," and the sequence of oligonucleotides is an important factor because it is involved in the recognition of target molecules.

P. 14 – 17

Confirmation of molecular weight, quantification, and concentration

→Confirmation of synthesized oligonucleotide product identity and examination of impurities by LC-MS precision mass spectrometry are important quality control tests.

UV-visible spectrophotometers are useful for simple oligonucleotide concentration verification and spectral confirmation.

P. 16 – 29

Separation of Impurities

Oligonucleotide Analysis by Ion Exchange Chromatography (IEX)

click here

benefits

- Short chain oligonucleotides can be separated based on the base unit.
- Oligonucleotides can be separated from impurities such as protecting groups used in the chemical synthesis process.
- It can be analyzed using mobile phases with high salt concentrations and a wide pH range.

Methods and Results

Sample	5'-TCTTGGTTACATGAAA-3' (16 mer) 5'-TCTTGGTTACATGAAAT-3' (17 mer) 5'-TCTTGGTTACATGAAATC-3' (18 mer) 5'-TCTTGGTTACATGAAATCC-3' (19 mer) 5'-TCTTGGTTACATGAAATCCC-3' (20 mer)
Conc., Volume	5 µmol/L, 4 µL
Preparation	Dilution in ultrapure water to the concentrations above.
Analytical Conditions	As shown in Table 1
Results	Target oligonucleotides in 20 mer and 4 sequences that were deleted from n-1 to n-4 on the 3' terminus of target were prepared as impurities derived from the synthesis. All of them were unmodified single-stranded DNA and synthesized by a solid phase synthesis (HPLC-purified). For ion-exchange chromatography, Figure 1 shows a chromatogram of a mixture of five-sequence oligonucleotide. Each oligonucleotide was separated by their length. Table 2 shows the relative standard deviations (% RSD, n = 6) of the retention time and area of the 16 - 20 mer oligonucleotide mixture, with RSD% less than 1% for both parameters. And then, a mixture of five oligonucleotides was prepared (four of them were HPLC-purified while 1 was only desalted) and compared with the mixture of all HPLC-purified nucleotides (Figure 2). The target oligonucleotides were completely separated from impurities such as free protecting groups and shorter length oligonucleotides.

Table 1 Analysis Conditions

System:	Nexera XS inert
Column:	Shim-pack Bio IEX Q-NP (100 mm × 4.6 mm I.D., 5 µm)
Mobile phase A:	10 mmol/L NaOH
Mobile phase B:	10 mmol/L NaOH containing 1 mol/L $NaClO_4$
Flow rate:	0.8 mL/min
Time program: (B Conc.)	25-32.5% (0-15 min) → 100% (15-20 min) → 25% (20-25 min)
Column temp.:	30 °C
Injection volume:	4 µL
Detection:	UV 260 nm (SPD-M40), UHPLC standard cell
Vial:	Shimadzu 1.1 mL sample vial

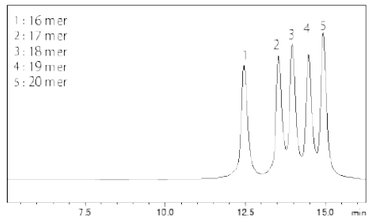


Figure 1 Chromatogram of oligonucleotides mixture

Table 2 Relative standard deviation (% RSD) of each component (n = 6)

Length(mer)	Retention time	Area
16	0.138	0.224
17	0.105	0.335
18	0.098	0.494
19	0.085	0.161
20	0.075	0.307

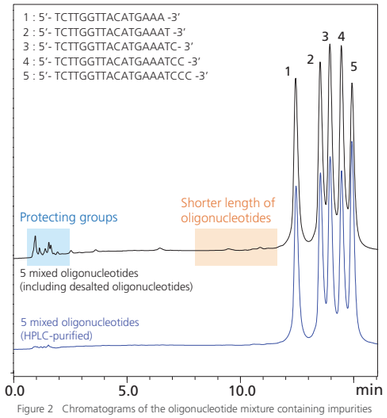


Figure 2 Chromatograms of the oligonucleotide mixture containing impurities

Conclusions

By using Nexera XS inert and Shim-pack Bio IEX, it is possible to reproducibly separate the desired oligonucleotide from impurities such as protecting groups generated during chemical synthesis or oligonucleotides with different chain lengths generated by incomplete synthesis.

Nexera XS inert

Features

The potential adsorption of an analyte onto wetted surfaces of UHPLC instruments poses some critical challenges when analyzing biomolecules. While elevated pressure tolerance is required to achieve optimal chromatographic separation when using small particle size columns, the inertness of the wetted surfaces is also of the utmost importance, as is resistance to corrosion due to the use of mobile phases with high salt concentrations and extreme pH values.
The Nexera XS inert system offers the ideal solution for the separation of biomolecules by combining the elevated pressure tolerance of a UHPLC system with complete inertness of the sample flow path, ensured by the absence of wetted metal surfaces and offering ultra-high resistance to corrosion.

EXPERIENCE
NEWFOUND CLARITY

Unconstrained Recovery and Sensitivity

Reduces sample loss due to adsorption to metal and achieves excellent sensitivity.

Clear Resolution without Restrictions

Improves peak shape and achieves excellent chromatographic separation.

Assured Reliability and Reproducibility

Reliable data for metal-adsorbing compounds with high reproducibility.

System Controller
SCL-40, CBM-40/40lite
Used to coordinate actions of the overall system, this controller offers intuitive operation that minimizes the stress involved in operating the system, from startup to shutdown.

Detector
SPD-40/40V/M40
Inert type cells for UHPLC analysis eliminate the risk of adsorption on the detector. Low-diffusion cell, which has 5 mm of optical path length, can also be selected.

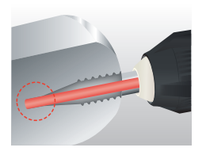
Solvent Delivery Unit
LC-40D XSi
Designed with corrosion-resistant non-stainless steel materials, it offers rugged, low-pulsation performance, advanced AI features and solvent blending capabilities. (optional).

Autosampler
SIL-40C XSi
This high-performance autosampler features nonmetal materials for all surfaces that contact liquids. That inhibits metal-adsorption of biomolecules.

UHPLC Inert Switching Valve
FCV-0206H2i/FCV-0607H2i
Designed with adsorption-inhibiting materials for all wetted surfaces.

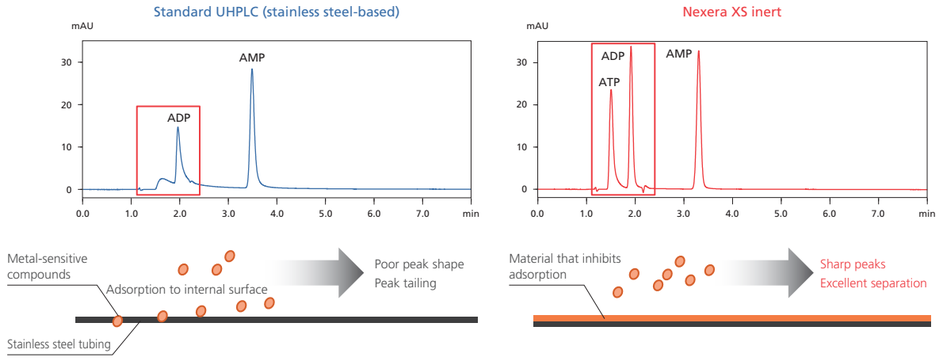
Finger Tight Fittings for Simple and Secure Connections

Nexera XS inert systems feature tubing connections with unique finger-tight fittings. They can achieve connections with up to 105 MPa of pressure capacity by finger-tightening and without creating any dead volume.



Resolution without Restrictions

The Nexera XS inert system is equipped with unique technology that ensures the complete inertness of the sample flow path. The system provides excellent peak shape and unsurpassed chromatographic separation by effectively inhibiting the adsorption of target compounds to internal surfaces.



Separation of Impurities

Oligonucleotide Analysis by Ion Pair Reverse Phase Chromatography

benefits

- Short chain oligonucleotides can be separated based on the base unit.
- By optimizing the composition of the mobile phase and the gradient conditions, the oligonucleotides were reproducibly separated by chain length

Methods and Results

Sample	Thymidylate (dTMP) 6, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 mer (HPLC - purified) 14-sequenced dTMP mixture
Conc., Volume	5 µmol/L, 5 µL
Preparation	Dilution in ultrapure water to the concentrations above.
Analytical Conditions	As shown in Table 1
Results of examination of analytical condition	The type and concentration of ion pairing reagent and the organic solvent were evaluated. Triethylamine (TEA) or dibutylamine (DBA) was selected as the ion-pairing reagent, and acetonitrile or methanol as the organic solvent. When TEA was used as an ion pairing reagent, multiple peaks overlapped in any combination with organic solvents, resulting in insufficient separation. When DBA was used as the ion pairing reagent, separation was improved compared to TEA. The concentration of DBA was adjusted from the previous conditions. Even when the concentration of DBA was low, oligonucleotides were separated with a good peak shape. Therefore, the concentration of DBA of 10 mmol/L was considered appropriate.
Results of separation	Based on the above results, 14-sequenced dTMP mixed samples were analyzed using the analytical conditions shown in Table 1. As a result, dTMP with short chain length was eluted in order and separated by chain length in units of base (Figure. 1). In addition, the relative standard deviation (% RSD) of the retention time and the area was less than 1 % when repeated analysis was performed six times (Table 2).

Table 1 Analysis Conditions

System:	Nexera XS inert
Column:	Shim-pack Scepter C18-120 [metal free] (150 mm × 4.6 mm I.D., 5 µm)
Mobile phase A:	10 mmol/L DBAA pH 6.0
Mobile phase B:	10 mmol/L DBAA pH 6.0 / Methanol = 20 : 80
Flow rate:	1.0 mL/min
Time program: (B Conc.)	45-65% (0-30 min) → 100% (30-35 min) → 45% (35-45 min)
Column temp.:	35 °C
Injection volume:	5 µL
Detection:	UV 260 nm (SPD-M40), UHPLC inert cell
Vial:	Shimadzu 1.1 mL sample vial

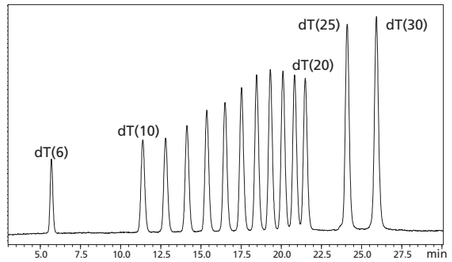


Figure 1 Chromatogram of 14-sequenced dTMP mixture

Table 2 Relative standard deviation (% RSD) of each component (n = 6)

Sample	Retention time	Area
dT(6)	0.079	0.760
dT(10)	0.048	0.493
dT(15)	0.038	0.443
dT(20)	0.030	0.867
dT(25)	0.028	0.767
dT(30)	0.028	0.757

Conclusions

By optimizing the column temperature, mobile phase composition, and gradient conditions, short oligonucleotides up to 30 mer in base units can be separated reproducibly for each chain length.

Nexera XS inert

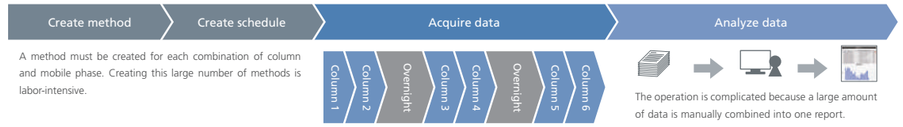
Features

Getting the right analytical conditions requires a lot of tests and data processing. Nexera XS inert can provide some great support by configuring several systems that are suitable for each purpose. Method Scouting System is a method development system based on Shimadzu's UHPLC technology. The combination with Method Scouting Solution dedicated control software achieves a fast and accurate method scouting workflow, offering excellent support for method development.

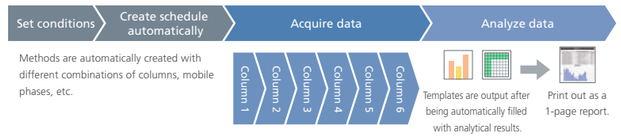
Automates Development of Analytical Conditions ~ Method Scouting ~

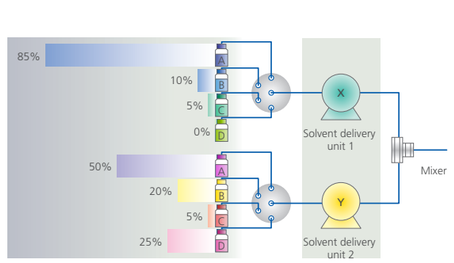
The process of considering which analytical conditions to specify for LC separation requires evaluating a huge number of possible combinations of columns, mobile phases, column temperatures, and other factors, which can be taxing on analytical personnel. The Nexera method scouting system offers functionality for automatically switching between multiple mobile phase conditions and columns for analysis. Consequently, it can be used to develop methods more efficiently by automating the process of optimizing mobile phase pH, salt concentration, or other analytical condition settings.

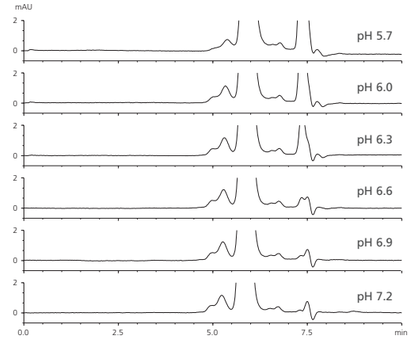
Previous Method



Method Scouting System + LabSolutions MD







Mobile Phase pH Monitor ~ pHM-40 ~

The pH monitor pHM-40 continuously monitors the pH of mobile phases to identify any changes in mobile phase pH in real time.

Tm Analysis

Tm Analysis of Nucleic Acid Drugs

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benefits

- The Melting Temperature (Tm) is calculated by two methods, the Average Method and the differential method.
- Multiple samples can be analyzed in parallel with eight microcells.
- It is possible to calculate thermodynamic parameter analysis as well as Tm measurement.

Methods and Results

Sample Forward Reverse	M13 primer 5'-CGACGTTGTAAAACGACGGCCAGGA-3' 5'-TCCTGGCCGTCGTTTTACAACGTCG-3'
Conc., Volume	The sample was prepared to have 0.5 to 1 Abs at 260 nm and a salt concentration of 100 mmol / L. 35 µL was added to a cell having an optical path length of 1 mm.
Preparation	The sample is an equal mixture of Forword and Reverse, heated at 95 °C for at least 10 minutes, and then annealed by cooling at a rate of 2 °C / min.
Analytical Conditions	As shown in Table 1
Results	Table 2 shows the results calculated by two types of calculation methods, the average method and the differential method. In the average method, tangents are drawn for each section selected in the pre-transition region and post-transition region, and the Tm is the intersection of the midlines of the two tangents and the absorbance curve. As shown in Fig. 1, the Tm is calculated by arbitrarily setting the pre-transition region and the post-transition region. In the differential method, a first-order differential operation is performed for each point within the set partition, and the position showing the maximum value is calculated as the Tm value. As shown in Fig. 2, the judgment area was set arbitrarily and the Tm value was obtained.

Table 1 Analysis Conditions

Spectrophotometer:	UV-2600i
Option:	TMSPC-8
Cell:	eight microcells
Optical pathlength:	1 mm
Wavelength:	260 nm
Blank Wavelength:	320 nm
Temperature range:	25 ~ 95 °C
Temperature rise:	1 °C
Ramp rate:	1 °C/ min

Table 2 Tm analysis results

Methods	Tm (°C)	
	Ascending process	Descending process
Average method	77.75	77.31
Differential method	77.92	77.85

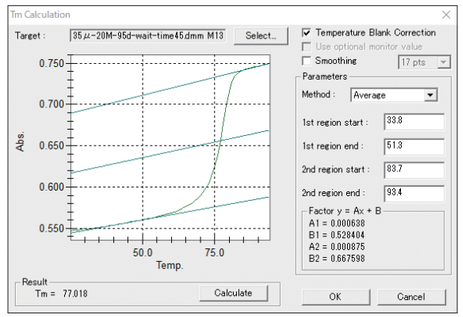


Figure 1 Example of analysis by the midline method

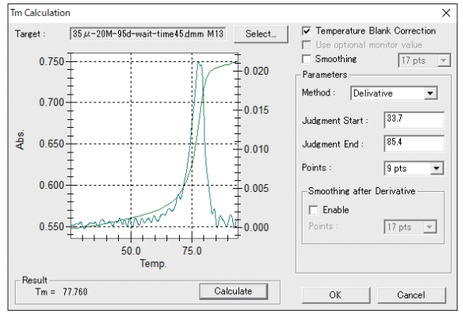


Figure 2 Example of analysis by differential method

Conclusions

Tm Analysis System can calculate Tm for double-stranded DNA and RNA for quality and stability evaluation. Also, by using Tm calculated at various concentrations, more detailed hybridization analysis is possible.

UV-2600i+TMSPC-8

Features

Tm analysis system, TMSPC-8, is connected to a spectrophotometer and measures the Tm of oligonucleotides by heating and cooling. The rate of temperature increasing/decreasing can be selected from 12 levels of ± 0.1, 0.2, 0.5, 1, 2 and 5 °C. There are 2 types of 8 micro-multi-cells with optical path length of 10 mm (minimum sample volume of 100 µL) and 1 mm (minimum sample volume of 10 µL), and these cells allow to measure 8 types of samples simultaneously in parallel.

Tm Analysis Software

As shown in Fig. 3, a typical melting curve has a region corresponding to a double-stranded or single-stranded structure at the low temperature side (pre-transition region) and at the high temperature side (post-transition region) with a transition region where the absorbance varies greatly. Tm is defined as the temperature at which the mole fractions of the double and single strands are equal.

One way to calculate Tm from a melting curve is called the average method. The baselines (tangents) in the pre- and post-transition regions are determined by the least squares method, and the intersection point between the median line of the two tangents and the absorbance curve is the Tm. For more details on this method, refer to "The Average Method Analysis" below. In addition, the method of calculating the first derivative of each point in the specified temperature range for the absorbance curve, and setting the Tm value as the maximum value of the first derivative is called the differential method.

The Tm analysis software calculates the Tm. In the average method, the Tm is calculated by setting a total of four points of the start point and the end point of the baseline in each of the before transition region and the after transition region.

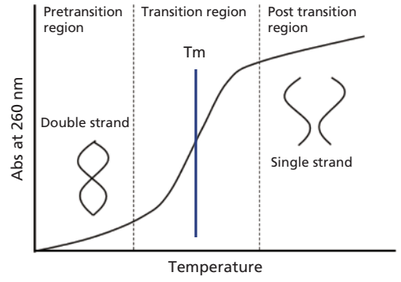


Figure 3 Example of Melting curve

The differential method calculates the Tm by setting the range of the temperature region. Since the absorbance changes depending on the temperature even when measuring only the buffer solution, this software has a temperature blank correction function that subtracts the buffer solution data from the data of the sample in advance. In order to further improve the accuracy of the analysis, it is also equipped with a two-wavelength correction function that determines the difference in absorbance by measuring at any two wavelengths.

The Average Method Analysis

The average method is analyzed on the assumption that a simple two-state equilibrium relationship is established between the duplex and the single strand. The absorbance can be expressed by the following equation, where ε_{ds} and ε_{ss} are the molar molecular absorption coefficients of the double and single chains, respectively.

$$Abs = (\varepsilon_{ds} \alpha + \varepsilon_{ss} (1-\alpha)) CL$$

C is the total strands concentration, L is the optical path length, and α is the mole fraction of the duplex. Tm is defined as the temperature at which alpha equals 1/2. In strict two-state treatment, ε_{ds} and ε_{ss} have constant values regardless of temperature, but they are not actually constant values and change in proportion to temperature (T), so it is necessary to introduce a term proportional to T.

$$\varepsilon_{ds} = a_{ds} + b_{ds}T \quad \varepsilon_{ss} = a_{ss} + b_{ss}T$$

The Tm analysis software uses these equations to calculate Tm values as follows:.

1.The user determines a start point and an end point of the baseline in the forward transition region and the backward transition region.
2.The software automatically calculates ads, bds, and bss by regression calculation.
3.The software automatically calculates the midline between these two baselines and calculates the intersection of the midline and the absorbance curve as the Tm value.

Thermodynamic Parameter Analysis

Tm Analysis and Thermodynamic Characterization of Oligonucleotide Therapeutics

click here

benefits

- The Tm can be used to determine the amount of change in the Gibbs free energy, an index of drug activity.
- Users get thermodynamic properties like entropy and enthalpy.
- Equipped with eight microcells, users can efficiently measure and analyze a large number of samples.

■ Methods and Results

Sample Forward Reverse	M13 primer 5'-CGACGTTGTAAAACGACGGCCAGGA-3' 5'-TCCTGGCCGTCGTTTTACAACGTCG-3'
Conc., Volume	2, 5, 9, 12, 20, 40, 60 µmol/L 2, 5 µmol/L :100 µL in a cell of 10 mm optical path length 9 ~ 60 µmol/L :10 µL in a cell of 1 mm optical path length
Preparation	Samples are prepared to the above concentrations using 66.7 mmol/L phosphate buffer containing 5 mol/L NaCl. Samples are then annealed by mixing equal amounts of Forward and Reverse, warming at 95 °C for more than 10 minutes, and cooling.
Analytical Conditions	As shown in Table 1
Results	We measured Tm for seven samples of different concentrations, and determined enthalpy change ΔH°, entropy change ΔS°, and Gibbs free energy change ΔG°. Changes in temperature and absorbance were plotted (Figure 1: Plot of 9 ~ 60 µmol/L for use in a 1 mm cell), and the Tm at each concentration were calculated from the resulting curves (Table 2). In addition, ΔG°, ΔH°, and ΔS° were calculated from the Tm of each concentration (Table 3).

Table 1 Analysis Conditions

Spectrophotometer:	UV-2600i
Option:	TMSPC-8
Cell:	eight microcells Pathlength 10 mm eight microcells Pathlength 1 mm
Wavelength:	260 nm
Blank Wavelength:	320 nm
Temperature range:	15 ~ 90 °C
Temperature rise:	1 °C
Ramp rate:	1 °C/ min

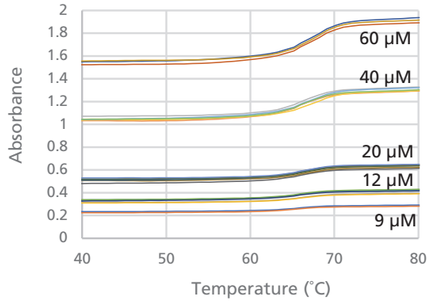


Figure 1 Plot of changes in temperature and absorbance at 9 ~ 60 µmol/L using a 1 mm cell

Table 2 Tm analysis results for each concentration

No.	concentration (µmol/L)	Tm (°C)
1	2	62.9
2	5	64.5
3	9	65.0
4	12	66.3
5	20	66.8
6	40	67.7
7	60	68.2

Table 3 ΔG°, ΔH°, ΔS° calculated from Tm values

Factor	Value
ΔH°	- 622 kJ/mol
ΔS°	-1792 J/ (mol·K)
ΔG°	- 107 kJ/mol

■ Conclusions

Tm Analysis System can obtain Tm and calculate thermodynamic indicators such as enthalpy change, entropy change and Gibbs free energy change. This result is an activity indicator for oligonucleotide therapeutics and is expected to contribute to the development of more effective drug discovery.

UV-2600i+TMSPC-8

Activity Index for Oligonucleotide Therapeutics ~ Thermodynamic Parameter Analysis ~

The Gibbs free energy is an indicator of the activity of oligonucleotide therapeutics.
The Gibbs free energy is the non-expanding work, which represents the maximum work used for binding between strands in oligonucleotides.
The higher the negative value, the stronger the binding force of the strands.

The Gibbs free energy change is described thermodynamically and statistically by the following equation

$$\Delta G = \Delta H - T\Delta S \qquad (1)$$

$$\Delta G = \Delta G^{\circ} + RT\ln K \qquad (2)$$

where K is the equilibrium constant, R is the gas constant, and Δ G ° is the standard Gibbs free energy.
When considering the equilibrium constant of a oligonucleotide, the oligonucleotide can be described as an equilibrium reaction between two molecules in which sense chain A and antisense chain B are associated in a 1:1 ratio.

$$A + B \leftrightarrow AB \qquad (3)$$

The double-stranded state mole fraction is denoted by α, and the total concentration of oligonucleotide is denoted by C. Because the concentrations of A and B [A] and [B] at complete dissociation are C/2, the equilibrium constant can be expressed as:

$$K = \frac{2\alpha}{c\,(1-\alpha)^2} \qquad (4)$$

It is in equilibrium when the temperature is Tm. And α = 1/2 and ΔG = 0, the equation (2) is expressed as follows.

$$\Delta G^{\circ} = RT_m \ln\frac{4}{c} \qquad (5)$$

ΔG° is the standard Gibbs free energy at 1 atm and 25 °C. Substituting expression (5) into expression (1) yields, the following relational expression:.

$$\frac{1}{T_m} = -\frac{R}{\Delta H^{\circ}}\ln\frac{4}{c} + \frac{\Delta S^{\circ}}{\Delta H^{\circ}}$$

$$= \frac{R}{\Delta H^{\circ}}\ln\frac{c}{4} + \frac{\Delta S^{\circ}}{\Delta H^{\circ}}$$

Based on the above, the enthalpy change ΔH ° of the standard state is determined from the slope of the straight line obtained by plotting the reciprocal of the Tm value (1/Tm) on the vertical axis and ln (C/4) on the horizontal axis, and the entropy change ΔS° of the standard state is determined from this slope and the y intercept.

Therefore, to determine ΔH° and ΔS° in duplex formation, it is necessary to prepare samples of various concentrations (C), determine the Tm of each sample, and plot 1/Tm against ln (C/4).

The Tm analysis software can calculate the Tm value for each concentration.

When analyzing by this method, the oligonucleotide concentration (C) should be as wide as possible (several tens of times or more). The combination of cells with optical path lengths of 10 mm and 1 mm makes it possible to measure data over a wide range of concentrations. In addition, the minimum required sample volume is 10 µL, which can be measured in small amounts.

In addition to the Gibbs free energy change ΔG°, which indicates the strength of binding force, the user can also know which function is dominant, which enables more effective drug discovery.

Sequence Confirmation

Simple Sequence Confirmation by MALDI-TOF MS

click here

benefits

- Rapid and easy nucleic acid analysis is possible with minimal pretreatment.
- MALDI-ISD allows simple sequencing of synthetic nucleic acids.
- Benchtop MS can be used with high maintainability and low running cost.

Methods and Results

Sample	5'-CTGAGACACTGAAGGTAGGA-3'
Conc., Volume	100 pmol/μL, 10 μL
Preparation	After activation with 1 N hydrochloric acid, approximately 200 μL slurry of cation exchange resin (Dowex 50 w 100-200 mesh, Dow chemical) washed with water was added to the Empty microspin column (BioRad), and the solution was removed by gentle centrifugation. Then, 10 μL of the sample was added to the Dowex cation exchange resin column prepared above, and the flow-through was recovered by centrifugation.
Mass spectrometry	1 μL of the collected solution was deposited onto a MALDI target plate, and the matrix solution (0.5 μL) was overlaid and dried. Measurements were performed in the positive-ion mode.
Results	As a result of MS analysis, a singly-charged ion was detected at *m/z* 6214.5 (Figure 1). The ISD spectrum of the nucleic acid was obtained as shown in Figure 2. The ISD in the positive-ion mode measurement of nucleic acid produces a very simple spectrum because of the preferential cleavage producing w-series ions (Figure 3). Sequence information of the nucleic acid can be easily obtained by assigning fragment ions of the ISD spectrum.

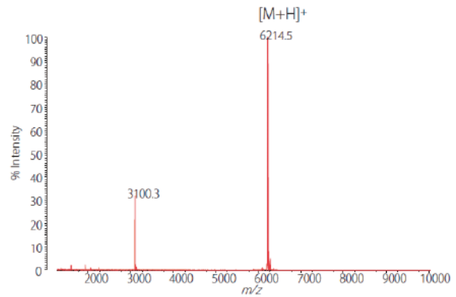


Figure 1 MALDI-TOF mass spectrum of the synthetic nucleic acid.

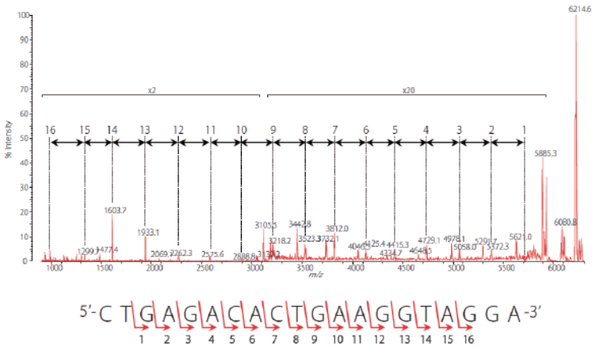


Figure 2 MALDI-ISD mass spectrum with the annotation of w-series ions of the nucleic acid.

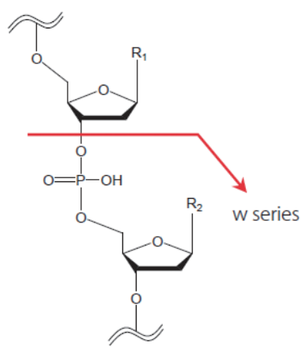


Figure 3 Cleavage site by ISD

Conclusions

By using the benchtop MALDI-TOF MS "MALDI-8020 " dedicated to positive linear mode, it is possible to easily confirm the molecular weight and sequence of nucleic acids.

MALDI-8020

Features

The MALDI-8020 is the latest in a long line of MALDI-TOF products from Shimadzu. It is used for quality control and profiling of peptides, proteins, polymers and oligonucleotides. In order to maintain equipment performance for a long period of time, even benchtop types use large diameter ion optics to reduce the risk of ion stain reduction. In addition, it is equipped with a UV laser quick automatic ion source cleaning function (TrueClean) that can clean the ion extraction electrode without dropping the vacuum. It also provides tools to centrally manage data and support strict adherence to 21 CFR Part 11.

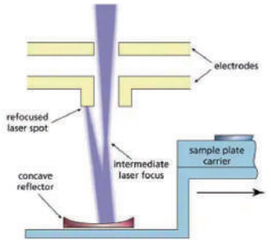
- Linear mode (positive ion) MALDI-TOF
- 200 Hz solid-state laser, 355 nm
- Load-lock chamber for fast sample introduction
- UV laser-based source cleaning (Pat. US 10340131)
- Small footprint/benchtop design
- Quiet operation (<55 dB)

Sample Target Solutions

Compatible with the FlexiMass series of microscope slide-format sample targets, these slides provide researchers with options depending on their experimental workflow.
The individually barcoded, single-use FlexiMass-DS slides provide a convenient solution for more routine or defined workflows. Ready-to-use, these disposable targets eliminate the need for cleaning and the risk of carryover. Alternatively, the reusable stainless steel FlexiMass-SR sample targets provide a cost-effective, longer-term solution to sample preparation.

Automated Source Cleaning TrueClean

To maintain instrument performance over time, the MALDI-8020 features wide-bore ion optics which minimize the risk of source contamination and provide a robust platform. TrueClean, an automated source cleaning function, cleans the extraction electrode in-situ quickly within 10 min by using UV laser without breaking instrument vacuum.



High Quality with Low-running Cost

For improving product performance and the longevity, components were carefully selected, which realized a robust platform capable of delivering outstanding performance in a small footprint. Simple design provides easy maintenance and contribute to maintaining the quality of analysis over time.

Database Management

Operating under the control of MALDI Solutions software, the software features a centralized, secure Microsoft SQL database which can be used to store everything from sample lists and acquisition Parameter Sets to acquired MALDI data. The system is managed by an administrator and customizable user profiles provide control over access to the database and operation of the instrument. Along with a full audit trail, the software provides the tools to help achieve 21 CFR part 11 regulatory compliance.

Sequence Confirmation & Molecular Weight Confirmation

Analysis of Oligonucleotide Therapeutics using MALDI-8030 and LCMS-9030

click here

benefits

- Confident characterization of oligonucleotides
- Elemental formula confirmation by ESI-QTOF
- Information of sequence by MALDI-ISD

Methods and Results

Sample	Phosphorothioated oligonucleotides, differing in the structure of the sugar constituents (Figure 1) LNA-Oligo (*m/z* 6711.6731) S-Oligo (*m/z* 6431.7240)
Conc., Volume	10 pmol/μL, 1 μL
Preparation	Dilution in ultrapure water to the concentrations above.
Molecular weight confirmation	An exact mass measurement in negative ion detection was conducted using a LCMS-9030. The solvent consisted of 50 mmol/L HFIP, 10 mmol/L DIPEA, and acetonitrile, was applied to the ESI at a flow rate of 0.2 mL/min. The MS range of the QTOF was set as m/z 500 to 3000. Deconvolution of ESI spectra was performed with ReSpect in LabSolutions Insight.
Results	Multiply-charged ions of the oligos distributed from $[M-4H]^{4-}$ to $[M-6H]^{6-}$ were observed (Figure 2, inset). Exact masses of two oligos were successfully obtained by deconvolution of the ESI spectra. *m/z* 6711.6733 was obtained from the ESI-MS of the LNA-Oligo (Figure 2), and m/z 6431.7241 from the S-oligo.
Internal sequence information analysis	ISD with negative ion detection was performed on a MALDI-8030, dual polarity benchtop linear MALDI-TOF MS. 3-HPA (3-hydroxypicolinic acid) and ammonium citrate were applied to MALDI-ISD measurement as matrix and additives, respectively. Matrix solution and samples were layered on a stainless MALDI plate. A transition from MS measurement to ISD in the instrument is quick and easy by simply increasing laser irradiation power.
Results	MALDI-ISD ladder sequence confirmation of the LNA-Oligo was shown in Figure 3. Fragment ions, denoted as a- and w-seiries ions, were assigned by matching against the theoretical average masses. w-ions derived from almost the whole oligo sequences were found in the spectra. Only one or two ions derived from the 3′-terminal units were missed due to an overlap with the matrix signals. In the case of the S-oligo, with the exception of two 5′-terminal units, almost a complete series of a-ions were detected. However, in the case of LNA-oligo, the detected a-ions corresponded to those derived from the internal sequence, indicating that a-ions support confirmation of the sequence.

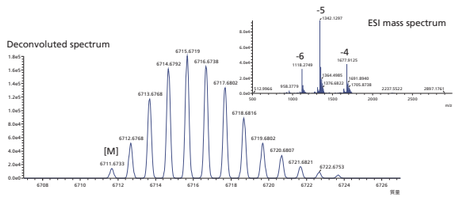


Figure 2 Exact mass measurement of LNA-Oligo

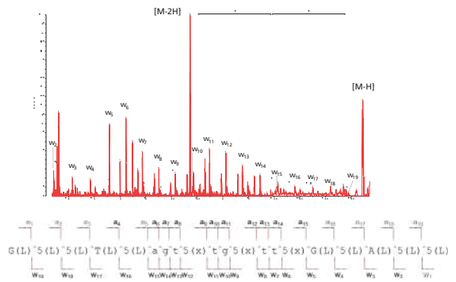


Figure 3 ISD of LNA-Oligo, and assignment of a- and w-series ions.

LNA-Oligo G(L)^5(L)^5(L)^T(L)^5(L)^a^g^t^5(x)^t^g^5(x)^t^t^5(x)^G(L)^5(L)^A(L)^5(L)^5(L)

S-Oligo g^5(x)^5(x)^t^5(x)^a^g^t^5(x)^t^g^5(x)^t^t^5(x)^g^5(x)^a^5(x)^5(x)

Legend N(L): LNA* (A, T, G) (small character): DNA
5(L): LNA^(5-mC DNA) 5(x): 5-methyl dC
*LNA: Locked Nucleic Acid ^ : Phosphorothioated

Figure 1 Sequences of oligonucleotides

Conclusions

Exact mass measurements using the LCMS-9030 resulted in doubtless consistency between the theoretical and observed masses. ISD using the MALDI-8030 resulted in mainly an internal sequence information, which is difficult to obtain with any MS/MS technique. The MALDI-ISD and ESI-QTOF are a useful combination to characterize oligonucleotide therapeutics.

LCMS-9030 / MALDI-8030

Features

The LCMS-9030 is a Q-TOF type mass spectrometer with two types of ion mass separation mechanisms: quadrupole and time-of-flight. While high-resolution and high-precision ESI mass spectrometers enable precise mass measurement of oligonucleotides, routine oligonucleotide sequence analysis using ESI-MS is still difficult, and complete internal oligo-sequences are rarely obtained using these general ESI-MS/MS techniques. The reason for this is that the Collision-induced dissociation (CID) used in ESI-MS/MS has difficulty in obtaining fragment ions that are effective for the internal sequence assignment of oligonucleotides.
On the other hand, in-source decay (ISD) using MALDI-TOF MS was reported as a useful method to conduct sequence analysis[1], although the instruments often employed do not have sufficient specification for exact mass measurement.

Reference
1) Shimizu H, Jinno F, Morohashi A, Yamazaki Y, Yamada M, Kondo T, Asahi S. *J Mass Spectrom*. 2012 Aug;47(8):1015-22.

LCMS-9030

New TOF Technologies

UFgrating (Pat. US 10020181)

Shimadzu's world-class manufacturing capability has enabled the ion acceleration electrode to be made with substantial mechanical strength. This grating is able to withstand the high voltages needed for ultrafast ion pulsing.

Traditional mesh electrodes for ion transmission lack mechanical strength, limiting acceleration voltage.

Shimadzu' s UFgrating has superior mechanical strength over conventional electrodes.
This unique grating structure makes it possible to apply a higher voltage.

UFgrating (Pat. US 10020181)

Ion accumulation in the collision cell, synchronized perfectly with short cycles of data acquisition, maximizes sensitivity.

UF-FlightTube (Patent Pending)

With excellent architecture, the UF-FlightTube prevents and withstands subtle deformations caused by temperature changes, affording stability of performance.

iRefTOF (Pat. US 8772708, 9490114)

A computationally ideal electrostatic field has become a reality. Meticulously manufactured plate electrodes are stacked to create a reflectron that compensates for the energy distribution of ions with no compromise in either resolution or sensitivity.

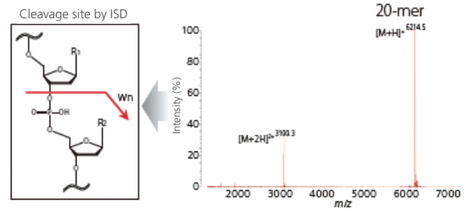
Key Technology

The LCMS-9030 uses newly patented technologies to deliver both high resolution and accurate mass, attributes essential for confident formula assignment and unknown identification. The high-efficiency ion guides, quadrupole, and collision cell enable high sensitivity for the detection of trace-level compounds. Unique UFgrating and iRefTOF technologies ensure ultrafast acceleration of ions into the flight tube (UF-FlightTube) and ideal reflection of those ions back to the detector. The result is high-speed data acquisition compatible with the high-throughput laboratory.

Internal Sequence Analysis by In-source Decay (ISD)

The ISD cleavage shown in the figure below enables easy sequence analysis even for MALDI-TOF MS without MS/MS capability.

MALDI-8030



Molecular Weight Confirmation

Negative Mode Analysis of Synthetic Oligonucleotides using the MALDI-8030 Dual Polarity Benchtop MALDI-TOF Mass Spectrometer

click here

benefits

- Simple analysis of oligonucleotides, in negative-ion mode to reduce adducts, on an affordable benchtop MALDI-TOF MS
- Quality spectra with good mass accuracy offers MALDI-TOF MS as an alternative to gel-Ethidium bromide detection post PCR

Methods and Results

Sample	5'-ATCTTTGGTTT-3' (Wild type / normal CFTR gene) Average mass : m/z 3656.43 5'-ATTGGTGT-3' (Phe508delCFTR mutated gene) Average mass : m/z 2758.85
Conc., Volume	100 µmol/L, 1 ~ 10 µL
Preparation	Samples were diluted with ultrapure water to the above concentration.
Matrix solution	Ammonium citrate dibasic was prepared at 5 mg/mL in 70:30 acetonitrile/water, which was used to prepare the 3-HPA matrix (45 mg/mL). Samples were pre-mixed with matrix (1:2) prior to spotting onto the MALDI target.
Positive ion mode	Desalting was performed using the Dowex cation exchange resin, which works through exchange of hydrogens for sodium and other salts.
Negative ion mode	No desalting was performed for the negative-ion mode analysis.
analysis	MALDI analyses were conducted on the MALDI-8030 in positive and negative ion modes using the desalted and non-desalted samples, respectively.
Results	Figure 1 shows the negative-ion mode MALDI mass spectra of the oligonucleotides which are representative of the different genotypes of cystic fibrosis: A) the subject has inherited the normal CFTR gene from both parents (wild type); B) the subject has inherited the mutated Phe508del CFTR gene from both parents (homozygote); C) the subject has inherited one normal CFTR gene and one mutated Phe508del CFTR gene (heterozygote). The insets in Figure 1A and Figure 1B show the corresponding oligonucleotide peaks obtained with the positive-ion mode analyses. As can be observed, even with desalting (cation exchange), some minor sodium and potassium adducts are still detected in positive-ion mode. In contrast, the corresponding spectra obtained in negative-ion mode are cleaner and free from salt adducts (no desalting is necessary). Figure 1A-C also shows the detection of the oligonucleotides is good using MALDI-TOF, as seen with the strong peak signals. The mass resolution also enables separation of the normal CFTR oligonucleotide and the Phe508del CFTR mutated oligonucleotide in the heterozygote (Fig 4C), therefore genotyping of all three outcomes for wild type, homozygote and heterozygote is easily possible.

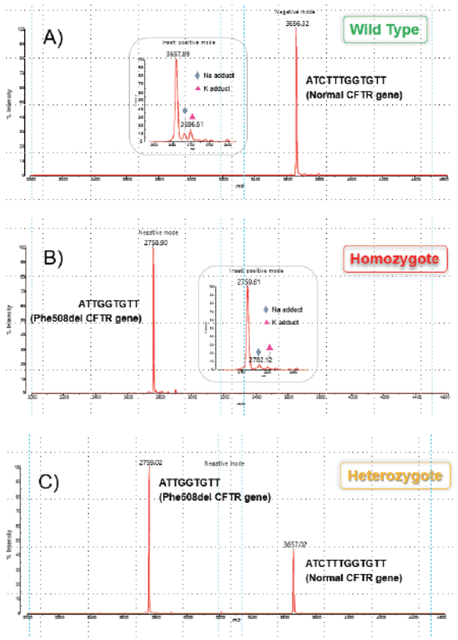


Figure 1 Negative-ion mode MALDI mass spectra of the oligonucleotides representative of the three different genotypes of cystic fibrosis

Conclusions

This application demonstrates the capability of the dual polarity MALDI-8030 to easily detect and resolve synthetic oligonucleotides. We demonstrated the benefits of the negative ion mode detection for eliminating the desalting sample clean up step in the analysis of oligonucleotides, while still producing good signal sensitivity. The overall analysis workflow is simple and faster than when performed via gel electrophoresis.

MALDI-8030

Features

The MALDI-8030 is the latest in a long line of MALDI-TOF products from Shimadzu. Instrument performance specifications are extended from those of the MALDI-8020 to cater for compounds best suited to analysis in negative ion mode. This dual-polarity, benchtop linear MALDI-TOF mass spectrometer delivers outstanding performance in a compact footprint, making it an ideal choice for today's increasingly demanding laboratories.
This high throughput and flexible analytical capabilities are ideal for quality control and profiling applications such as peptides, proteins, polymers and oligonucleotides.

- Dual mode (positive / negative ion) MALDI-TOF
- 200 Hz solid-state laser, 355 nm
- Load-lock chamber for fast sample introduction
- UV laser-based source cleaning (Pat. US 10340131)
- Small footprint/benchtop design
- Quiet operation (<55 dB)

Sample Target Solutions

Compatible with the FlexiMass series of microscope slide-format sample targets, these slides provide researchers with options depending on their experimental workflow.
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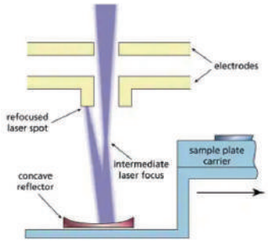
To maintain instrument performance over time, the MALDI-8030 features wide-bore ion optics which minimize the risk of source contamination and provide a robust platform. TrueClean, an automated source cleaning function, cleans the extraction electrode in-situ quickly within 10 min by using UV laser without breaking instrument vacuum.



High Quality with Low-running Cost

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Database management

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Molecular Weight Confirmation & Quantitation

Molecular Weight Determination and Quantitation of Oligonucleotide Therapeutics Using Quadrupole Time-of- Flight Mass Spectrometer LCMS-9030

click here

benefits

- High-resolution mass spectra provide more accurate molecular weight confirmation.
- Achieves stable mass accuracy without frequent mass calibration.

Methods and Results

Sample	5'-mG-mC*-mC*-mU*-mC*-dA-dG-dT-dC*-dT-dG-dC*-dT-dT-dC*-mG-mC*-mA-mC*-mC*-3' m: 2'-O-(2-Methoxyethyl) nucleoside (2-MOE) * : 5-methylated derivatives of C and U D : 2'-deoxyribonucleoside Monoisotopic mass: 6431.7239
Conc., Volume	1 ~ 1000 ng/mL, 5 µL
Preparation	Dilution in ultrapure water to the concentrations above.
Analytical Conditions	The HPLC and MS analysis conditions are shown in Table 1. In this analysis, HFIP (1,1,1,3,3,3-Hexafluoro-2-propanol) and DIPEA (N, N-diisopropylethylamine) were used in the mobile phase to allow highly sensitive measurement to be performed.
Results	Figure 1 shows the mass spectrum extracted from the scan mode data. In the mass spectrum, multi-charged ions such as those at *m/z* 1071.6, 918.4 and 803.5 were detected. The *m/z* values of these multi-charged ions were deconvoluted and Figure 2 shows the results of molecular mass calculation. As shown in the deconvolution spectrum, the monoisotopic mass was 6431.72 with a mass error of 3 mDa (0.05 ppm). The calibration curve is shown in Figure 3 insert, The calibration curve was prepared from 1 - 1000 ng/mL. The correlation coefficient (r^2) was 0.996. Figure 3 shows representative chromatograms obtained using multiple reaction monitoring (MRM) mode. The octavalent ion at *m/z* 803.4626 was selected as a precursor ion. The product ion at *m/z* 94.9358 (PSO_2^-) was used for quantitation.

Table 1 Analysis Conditions

[HPLC conditions] (Nexera)	
Column:	Shim-pack Scepter C18 (75 mm × 2.0 mm I.D.,1.9 µm)
Mobile phases:	A)50 mmol/L HFIP and 10 mmol/L DIPEA
	B)Acetonitrile
Gradient Program	B% (0-0.5 min) – 15%(0.5-6 min)
Flow rate :	0.2 mL/min
Column Temp. :	50 °C
Injection volume :	5 µL
[MS conditions] (LCMS-9030)	
Ionization:	ESI(Negative mode)
Probe Voltage:	-3 kV
Mode:	Full-scan (m/z 500-3000)
	MRM(803.4626>94.9358)
Nebulizing gas flow:	3.0 L/min
Drying gas flow:	10.0 L/min
Heating gas flow:	10.0 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	350 °C

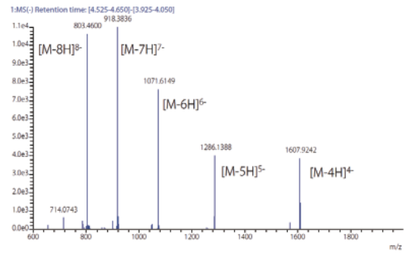


Figure 1 Mass Spectra of Oligonucleotide Therapeutics

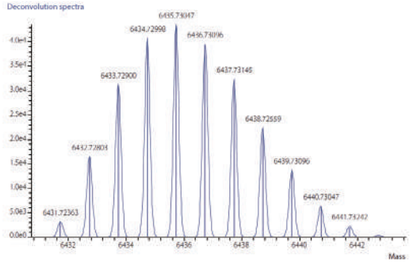


Figure 2 Deconvolution spectra

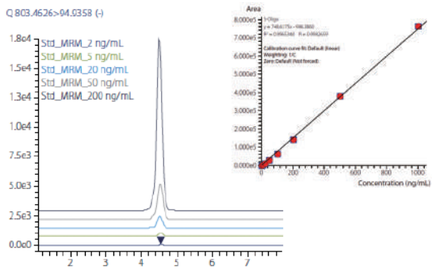


Figure 3 MRM Chromatograms of Oligonucleotide Therapeutics and Calibration Curve

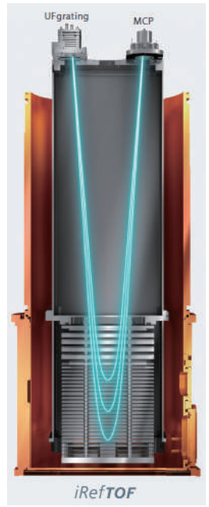
Conclusions

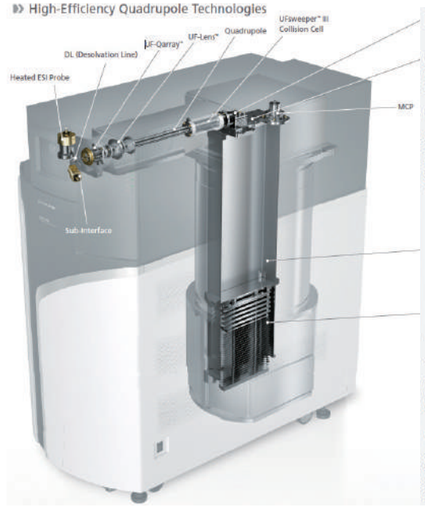
Accurate mass spectrometry using the Q-TOF mass spectrometer LCMS-9030 determined the molecular mass with an error of 0.05 ppm. Additionally, linearity was observed within a range of 1 - 1000 ng/mL.

LCMS-9030

Features

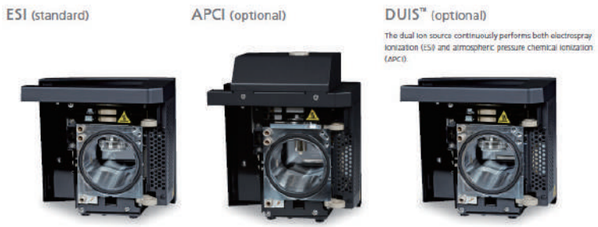
LCMS-9030 is a quadrupole time-of-flight (Q-TOF) mass spectrometer with two types of ion mass separation mechanisms: quadrupole and time-of-flight. Unique technologies abound, such as technology to improve ion utilization efficiency, processing technology to produce high-strength fine grid electrodes, high-precision temperature control, and optimization of potential distribution, enabling data acquisition with both high sensitivity and high resolution while maintaining stable mass accuracy.
Since oligonucleotide therapeutics have a molecular weight of 6000 or more at 20 bases, high-precision mass spectrometers such as Q-TOF mass spectrometers are used to confirm the molecular weight.





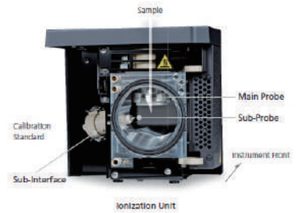
Simple Ionization Unit

In addition to the standard ESI ionization unit, optional APCI and Dual Ion Source (DUIS) probes are available for the LCMS-9030 to meet various analytical needs. Shimadzu's DUIS offers an efficient combination of ESI and APCI ionization capabilities.



Calibrant Delivery System (CDS)

The CDS allows calibration standards to be introduced via a separate ionization probe that functions independently from the main probe. This optional sub-ionization unit is installed in the main probe housing and is available for all probe types (ESI/APCI/DUIS). By having two probes in one system, high-concentration calibration standards can be introduced only when needed without switching flow lines, and are kept in isolation from the sample stream, eliminating contamination.



Molecular Weight Confirmation & Quantitation

Quantitation and Confirmation of Molecular Weight of Oligonucleotide Therapeutics

click here

benefits

- High sensitivity quantitation and molecular weight confirmation are possible with one system.
- Ease of maintenance helps minimize analytical downtime.

Methods and Results

Sample	5'-mG-mC*-mC*-mU*-mC*-dA-dG-dT-dC*-dT-dG-dC*-dT-dT-dC*-mG-mC*-mA-mC*-mC*-3' m: 2'-O-(2-methoxyethyl) nucleoside (2'-MOE) * : Indicates 5-C or 5-U methylation D : 2'-deoxynucleoside Average mass : 6436.39
Conc., Volume	1 ~ 300 ng/mL, 2 µL
Preparation	Dilution in ultrapure water to the concentrations above.
Analytical Conditions	Table 1 shows the HPLC and MS method conditions. An ionpair reagent is generally used in reversed phase separation of oligonucleotides.
Results	Fig. 1 shows a representative chromatogram acquired in MRM mode and the calibration curve. The calibration curve was prepared from 1 to 300 ng/mL. Fig. 2 shows the mass chromatogram measured in scan mode. In the mass chromatogram, hexavalent, heptavalent, and octavalent ions were selected. Figure 3 shows the mass spectrum. Pentavalent to octavalent ions were detected. As shown in the deconvolution results, the estimated molecular weight was determined to be 6436.37 (theoretical value: 6436.39).

Table 1 Analysis Conditions

[HPLC conditions] (Nexera)			
Column:	C18 Column (50 mm × 2.1 mm I.D., 2.5 µm)		
Mobil phases:	A) 50 mmol/L HFIP and 10 mmol/L DIPEA B) Acetonitrile		
Gradient Program:	B Conc. 5%(0-0.5 min) – 15%(0.5-3 min)		
Flow rate:	0.2 mL/min		
Column Temp.:	60 °C		
Injection volume:	2 µL		
[MS conditions] (LCMS-8060)			
Ionization:	ESI(Negative mode)		
Probe Voltage:	-4 kV		
Mode:	Full-scan (*m/z* 500 - 2000) MRM(*m/z* 803.5 > 95.0)		
CID gas:	330 kPa		
Nebulizing gas flow:	3.0 L/min	Drying gas flow:	8.0 L/min
Heating gas flow:	12.0 L/min	DL Temp.:	300 °C
Heat Blok Temp.:	450 °C	Interface Temp.:	250 °C

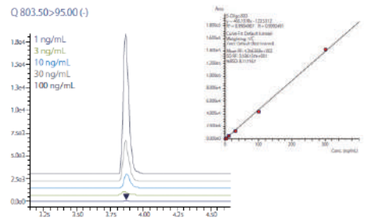


Figure 1 MRM Chromatograms of Oligonucleotide Therapeutics and Calibration Curve

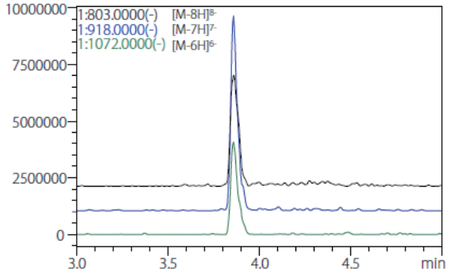


Figure 2 Mass Chromatogram of Oligonucleotide Therapeutics

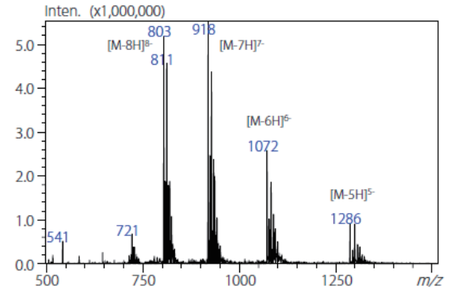


Figure 3 Mass Spectra of Oligonucleotide Therapeutics

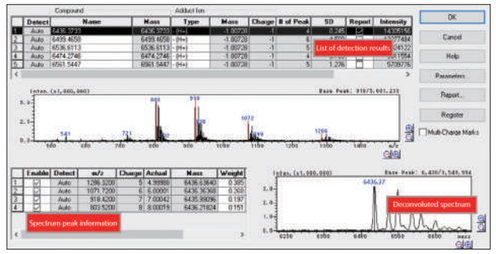


Figure 4 Results of Multivalent Ion Analysis

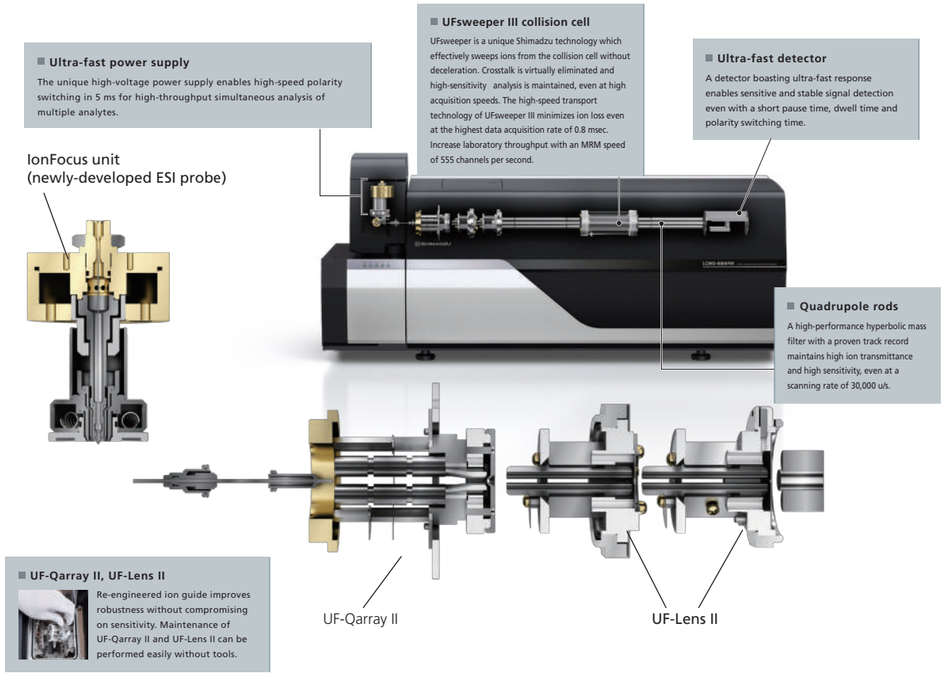
Conclusions

High-speed, high-sensitivity triple-quadrupole mass spectrometry enables molecular weight estimation and quantitative analysis of oligonucleotide therapeutics

LCMS-8060NX

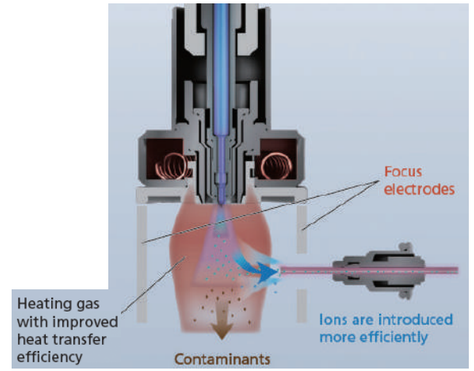
Features

LCMS-8060 NX is the culmination of Shimadzu's expertise in triple quadrupole mass spectrometry which boasts increased robustness while maintaining the high sensitivity and ultra-fast acquisition speeds that are the hallmark of Shimadzu's technology.
In response to work-flow changes and the need for cost reductions in recent years, the market for LC-MS systems has diversified, and there is a demand for highly efficient systems that are robust and easy to operate in addition to being highly sensitive. Therefore, LCMS -8060 NX adopts a unique new ion source, the Ion Focus unit (Patent No. 6593548, US 10546740), that efficiently introduces ions into the mass spectrometer and removes unnecessary neutrals. In addition, technologies such as UF-Qarray II and UF-Lens II, which have been reengineered to be even more resistant to contamination, are incorporated to minimize equipment downtime and improve laboratory operational efficiency.



Ion Focus

The ESI (Electrospray ionization) method, which is widely used as an ionization method for LC/MS, applies a high voltage to a nebulized sample spray, creating charged droplets. Desolvation results in gas phase ions which are introduced into the mass spectrometer. However, neutrals and matrix can enter the mass spectrometer, causing contamination and a decrease in sensitivity. If the spray position is moved further from the MS inlet in order to mitigate contamination, both neutral contaminants and ions of interest are effected, resulting in reduced sensitivity.
The new Ion Focus unit has been designed to solve this problem. The focus electrode for ion transport allows only ions of interest to be drawn into the mass spectrometer. As a result, unnecessary solvents and matrix can be removed, making it possible to achieve both high sensitivity analysis and an extremely robust system.



Molecular Weight Confirmation & Quantitation

Quantitative Analysis and Determination of
Molecular Weight by Triple Quadrupole Mass Spectrometry

click here

benefits

- High sensitivity quantitation and molecular weight confirmation are possible with one system.
- Ease of maintenance helps minimize analytical downtime.

Methods and Results

Sample	5'-pU CGAAGUAUUCCGCGUACG dTdT-3' Mw: 6646.0 (average mass) 5'-pC GUACGCGGAAUACUUCGA dTdT-3' Mw: 6669.0 (average mass)
Conc., Volume	1 ~ 10000 fmol/L, 10 µL
Preparation	Dilution in ultrapure water to the concentrations above.
Analytical Conditions	As shown in Table 1
Results	Calibration curves for AS-Oligo and SS-Oligo were obtained from 1 fmol to 10,000 fmol. SIM chromatograms for the injection volumes of 1 fmol (limit of detection, LOD) and 5 fmol (limit of quantitation, LOQ). Figure 1 shows the calibration curves. The coefficient of determination (R^2) was 0.997 for AS-Oligo and 0.995 for SS-Oligo. Figure 2 shows the SIM chromatogram of a mixed solution of SSOligo and AS-Oligo (100 fmol each). Using ion pair chromatography, retention times were 6.88 min and 6.94 min, respectively, and resolution R was 0.3. Figures 3 and 4 show the mass spectra of the two components. Ions with valences of 4 to 9 were detected in both SS-Oligo and AS-Oligo. Deconvolution results estimated the molecular weights of AS-Oligo and SS-Oligo at 6,645.6 and 6,667.1, respectively, with corresponding errors from the average molecular weights at 0.4 Da and 1.9 Da.

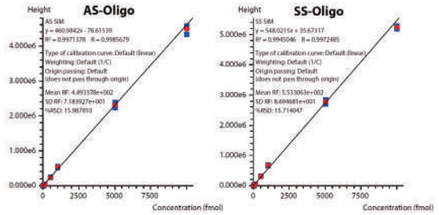


Figure 1 Calculation Curves Obtained from SIM Chromatograms

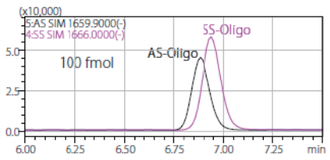


Figure 2 SIM Chromatogram

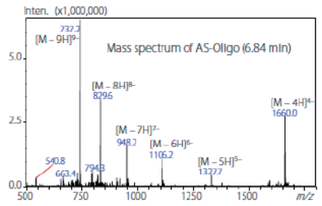


Figure 3 Mass Spectra of AS-Oligo

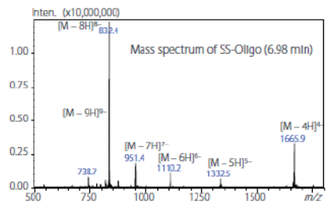


Figure 4 Mass Spectra of SS-Oligo

Table1 Analysis Conditions

[HPLC conditions] (Nexera)	
Column:	Commercially available C18colmn (100 mm × 2.1 mmI.D., 1.7 µm)
Mobile phases:	A) 200 mM HFIP*1 and 7.5 mM TEA*2/water B) Methanol
Grandient Program:	B conc. 4%(0 min) – 20%(8.0 min)
Flow rate:	0.2 mL/min
Column Temp.:	75 °C
Injection volume:	10 µL
[MS conditions] (LCMS-8060)	
Ionization:	ESI(Negative mode)
Probe Voltage:	-3 kV
Mode:	Q3scan(*m/z*500-1800) SIM *m/z* 1666.0(SS), *m/z*1659.9(AS)
Nebulizing gas flow:	3.0 L/min
Drying gas flow:	5.0 L/min
Heating gas fow:	15.0 L/min
DL Temp.:	250 °C
Heat Block Temp.:	500 °C
Interface Temp.:	350 °C

*1 1,1,1,3,3,3-Hexafluoro-2-propanol
*2 Triethylamine

Conclusions

Together with accurate quantitative analysis of an siRNA type oligonucleotide using the LCMS-8060 and SIM, molecular weight determination can be made with an error of just a few Da using deconvolution calculation capabilities.

LCMS-8060

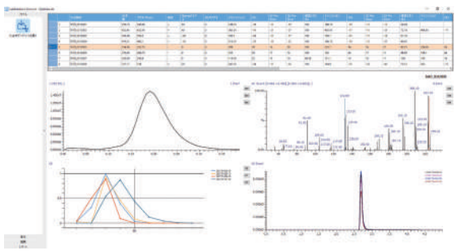
Features

LCMS -8060 is a triple quadrupole mass spectrometer that combines high speed and high sensitivity.The mass range is m/z 2 ~ 2000, and molecular weight estimation of oligonucleotide therapeutics is possible using the deconvolution function of LabSolutions LCMS software.
Triple-quadrupole mass spectrometers, which have high sensitivity and a wide dynamic range, are commonly used for quantitative analysis such as pharmacokinetic analysis of drugs, but also offer qualitative capabilities. Precise mass spectrometry such as MALDI-TOF and Q-TOF LC/MS are frequently used for accurate mass molecular weight determination.

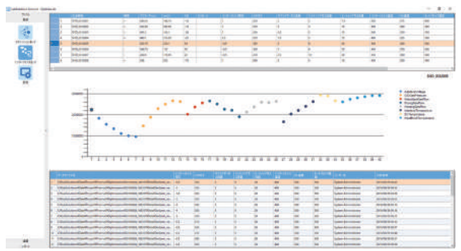
Software Solutions from Acquisition to Data Review
LabSolutions Connect MRM / LabSolutions Insight

Automatic optimization of MS conditions

MRM parameters (precursor ion m/z, product ion m/z, voltages) and ion source parameters (gas flow rate, temperature) are automatically optimized. Just one round of comprehensive optimization maximizes sensitivity, taking into consideration polarity, adduct ions, charge number etc. The results of this process can be viewed on a graph using the data browser function. The MRM optimization results screen simultaneously displays a chromatogram, a spectrum, and each voltage. From the MRM optimization results screen, check how the signal intensity changes with variations in each parameter.



MRM optimization
Review screen for MRM optimization simultaneously displays precursor ion, product ion, and voltage results..



Ion source optimization
Review screen for source optimization graphically displays the successive results of each parameter modication.

Quantitation & Spectrum Confirmation

Quantitation of Double-stranded DNA
– Trace Measurement Using TrayCell and Nano Stick –

click here

benefits

- It enables spectrum acquisition with ultrafast scanning at a maximum speed of 29,000 nm/min.
- The use of TrayCell (manufactured by Hellma Analytics) and Nano Stick (manufactured by SINCO) enables measurement from a sample volume of as little as 3 µL.
- The quantitative value of oligonucleotide concentration can be calculated by using the oligonucleotide quantification function in Biomethod.

Methods and Results

Sample	Double-stranded DNA
Conc., Volume	1 ~ 300 ng/mL, 2 µL
Preparation	Dilution in ultrapure water to the concentrations above.
Analytical Conditions	As shown in Table 1
Method for measuring Double-Stranded DNA using TrayCell	We prepared ds DNA and prepared standard samples at 27.5, 55, 110, 220, and 440 ng/µL (ultrapure water was used as the dilution solution). For samples with unknown concentrations, we prepared the same DNA by ethanol precipitation. The TrayCell can be changed to optical path lengths of 1.0 mm and 0.2 mm by using two different lids. In this test, we used a lid with an optical path length of 1.0 mm, dropped 4 µL, and measured under the conditions shown in Table 1 (Fig. 1).
Method for measuring Double-Stranded DNA using Nano Stick	We prepared standard and unknown samples of ds DNA as well as the above TrayCell. We also set the anallysis conditions as shown in Table 1. The optical path length of the Nano Stick was 0.5 mm, and the sample volume was measured at 3 µL (Fig. 2).
Results	Figures 3 and 4 show the calibration curve and UV spectrum results when measured using TrayCell and Nano Stick. Both results indicate a highly linear calibration curve. We repeatedly measured 440 ng/µL 10 times. As a result, the correlation function and CV value were calculated, and it was confirmed that the measurement was performed accurately.

Table 1 Analysis Conditions

Wavelength (calibration curve):	260 nm, 320 nm
Wavelength range:	220 nm ~ 330 nm
Scan Speed :	Low
Sampling Pitch:	1 nm

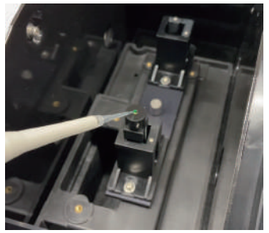


Figure 1 TrayCell Appearance

1. The sample is placed in the central silver region.

2. set the lid on

3. Set the cell holder with Traycell

4. After measurement, wipe the sample with a Kim wipe.

Figure 2 What the Nano Stick Looks Like and How To Use It

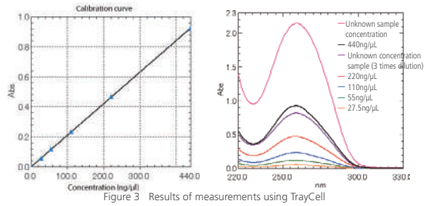


Figure 3 Results of measurements using TrayCell

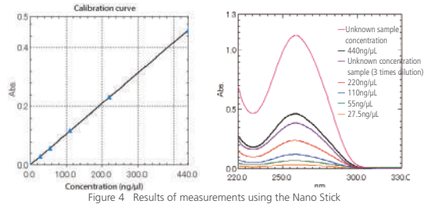


Figure 4 Results of measurements using the Nano Stick

Conclusions

This application news has shown that the UV-1900i, TrayCell, and Nano Stick can be used to make accurate and simple measurements even for small samples of the order of several µL.

UV-1900i

Features

The UV-1900i spectrophotometer features a space-saving and ergonomic hard design. It is equipped with a color touch panel and adopts a user interface (UI) that realizes "Easy to Operate, Obtain Answers Easily and Rapidly". In addition, Six types of measurement conditions are built in: 1. Oligonucleotide quantification, 2. Lowry method, 3. BCA method, 4. CBB (Bradford method), 5. Biuret method, and 6. UV absorption method. It also has a screen shot function on the operation panel, so you can easily extract measurement results without connecting to a PC. A 10 mm square cell requires a sample volume of approximately 4 mL, but the TrayCell or Nano Stick can be used to measure trace amounts of about 2 µL to 4 µL.

High Performance to Meet Diverse Needs

High Speed Scan	Spectra can be acquired as fast as 29,000 nm/min. Ultra-fast scan is effective in tracking chemical reactions in a short time. In addition to the absorbance change at specified wavelengths, spectra can also be acquired in a short time with the UV-1900i. Therefore, more detailed behavior can be investigated by observing spectra with the UV-1900i.
Low Stray Light	Stray light is at 0.5 % max. (198 nm), making accurate measurements are possible up to the vicinity of 2 Abs even in the ultraviolet region. In addition, high-concentration samples can be quantified accurately.
High Reproducibility and Repeatability Accuracy	High repeatability of less than 0.0002 Abs reduces variation in measurement results. And it enables more accurate quantitative analysis and detection of lower concentrations of samples.
A Diversity of Measurement Modes	UV-1900i features 6 measurement modes: Photometric, Spectral, Quantitative, Kinetics, Timecourse, and BioMethod.

Full Support for Pharmacopeia, GLP/GMP, FDA 21 CFR Part 11 and Other Regulations

Instrument Validation Functions Compliant with JP, USP, and EP	This instrument can not only run checks for nine JIS items, but also those stipulated in the Japanese Pharmacopoeia (JP), United States Pharmacopeia (USP), and the European Pharmacopoeia (EP). Naturally, the hardware is also compliant with the specifications required by each Pharmacopeia. In addition, the check conditions can be saved. As a result, once the conditions are saved, checks can be performed easily just by calling them up as needed. Check results can also be saved.
Resolution of 1 nm, the Highest in its Class	In addition to achieving a resolution of 1 nm, the highest in its class, by using a monochromator with a Czerny-Turner mounting, the UV-1900i also features a compact, bright optical system. The instrument is more than capable of meeting the wavelength resolution required in the European Pharmacopoeia.
Improved Security Functions	An external control security function has been added to provide more support for compliance with regulations. Three user authority levels, "Administrator", "Developer", and "Operator", can be set for instrument users.
Support for Regulations and Guidelines	Ensuring the integrity of data (database management), including the user management, user authority management, and data audit trails required for compliance with FDA 21 CFR Part 11, PIC/S GMP guidelines, and other ER/ES regulations is possible. LabSolutions DB UV-Vis or UVProbe / LabSolutions DB System allows for data management and user management with a database. Compliant with ER/ES regulations, the system is optimally configured for customers using a PC. LabSolutions CS UV-Vis or UVProbe / LabSolutions CS System (Network System) is optimally configured for customers who want to manage data on a server together with LC and GC data for ER/ES compliance.

Quantitation & Spectrum Confirmation

Quantitation of Nucleic Acid Using BioSpec-nano

click here

benefits

- It can be used to measure the concentration of extracted double-stranded DNA and to check the degree of purification.
- It is possible to measure from a minimum sample capacity of 1 µL.
- It is equipped with an automatic wiping function that achieves low carryover.

Methods and Results

Sample	Double-stranded DNA
Conc., Volume	As shown in Table 1
Preparation	Samples were prepared using Tris-EDTA (TE) buffers at the concentrations shown in Table 1.
Analytical Conditions	Samples were measured to 10 times of optical density (OD) at 260 nm at each optical path length (Table 1).
The result of optical path length 0.2 mm	The correlation coefficient between the measured value and the reference value was 0.999 (Figure 1). When the concentration was 250 ng/µL or more, the reproducibility of measurements was 1.4% or less for CV values (%) and -5.4% to 2.8% for OD errors (%).
The result of optical path length 0.7 mm	The correlation coefficient between the measured value and the reference value was 0.999 (Figure 2). When the concentration was 70 ng/µL or more, the reproducibility of measurements was 1.4% or less for CV values (%) and -8.6% to 4.4% for OD errors (%).
The result of a 5 mm optical path length cell	The correlation coefficient between the measured value and the reference value was 0.999 (Fig. 3). When the concentration was 70 ng/µL or more, the reproducibility of measurements was 0.6% or less for CV values (%) and -1.6% to 3.6% for OD errors (%).

Table 1 Analysis Conditions

Opticall path length 0.2 mm	Concentration	50 ~ 3700 ng/µL
	Volume	1 µL
Opticall path length 0.7 mm	Concentration	15 ~ 1000 ng/µL
	Volume	2 µL
5 mm pathlength cell (Option)	Concentration	2 ~ 150 ng/µL
	Volume	2 mL

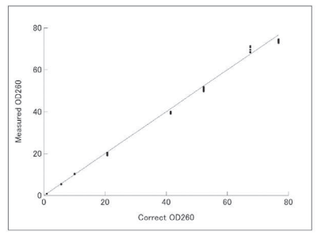


Figure 1 The result of optical path length 0.2 mm

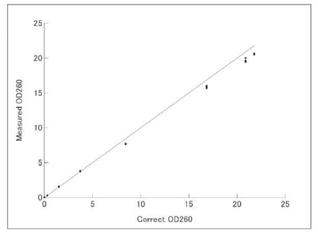


Figure 2 The result of optical path length 0.7 mm

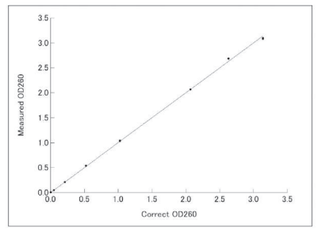


Figure 3 The result of a 5 mm optical path length cell

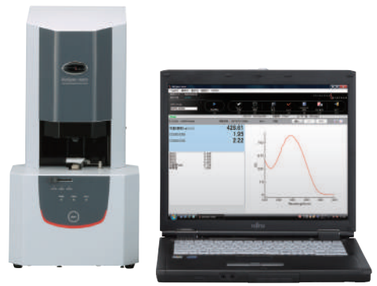
Conclusions

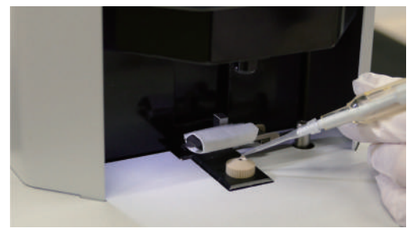
Bipspec-nano enables nucleic acid quantification with excellent photometric linearity, reproducibility, and accuracy at optical path lengths of 0.2 mm and 0.7 mm with sample volumes of 1 ~ 2 µL, respectively.

BioSpec-nano

Features

The BioSpec-nano is ideal for measuring precious biological samples, such as nucleic acids and proteins, as it can measure as little as 1 ~ 2 µL without using a cell (cuvette).
The measurement is started simply by dropping the sample into the dropping position (target) and pressing the start button. In addition, the automatic wiping function does not require sample wiping after measurement. While maintaining high correlation with standard type spectrophotometer (Cell Measurement, Double Beam), high reproducibility and measurement accuracy have been achieved. The optical path length can be selected from 0.2 mm and 0.7 mm depending on the sample concentration. In addition, if the option is purchased, measurement using a cell with an optical path length of 5 mm (sample volume of 2 mL) is also possible.
The BioSpec-nano is equipped with a variety of quantification modes, including nucleic acid quantification, protein quantification, OD value display at specified wavelengths, and can be used in a variety of applications.





You can measure samples one after another while holding the micropipette in your hand.

Automatic wiping function prevents forgetting to wipe

We measured 3 µL of double-stranded DNA with an optical path length of 0.7 mm and 578 ng/µL, followed by one wiping operation, and then measured TE buffer to determine carryover (%) (Equation 1). The wiping operation is set to be performed once after the sample or TE buffer measurement. As a result of performing 60 sets of double-stranded DNA measurement → wiping → TE buffer → wiping in 1 set, the carryover (%) was kept at 0.3% or less, and it was confirmed that the remaining sample amount was very low when automatic wiping was used.

The device automatically sets / measures / wipes the optical path length.

Carryover (%)

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

Various quantitative modes applicable to various samples

Quantitative mode	: This mode is used to quantify RNA, dsDNA, ssDNA, OligoDNA, etc. and calculate nucleic acid concentration, OD ratio (OD 260/280, OD 260/230).
Labeled nucleic acid quantification	: This mode is used to quantify nucleic acids labeled with Cy3 and others (RNA, dsDNA, ssDNA, OligoDNA) to calculate nucleic acid concentration, label concentration, nucleotide concentration, and labeling rate.
Protein quantification	: This mode finds the protein concentration from the absorbance at 280 nm. In addition, it is possible to determine the label concentration and labeling rate for labeled proteins as well as nucleic acids.
OD display for specified wavelength	: This mode displays the OD value for any specified wavelength, up to a maximum of 8 wavelengths.

TORAST-H Series

This is a series of high-end consumables that Shimadzu GLC has evaluated and verified using the latest technology.

Original Low Adsorption Vial

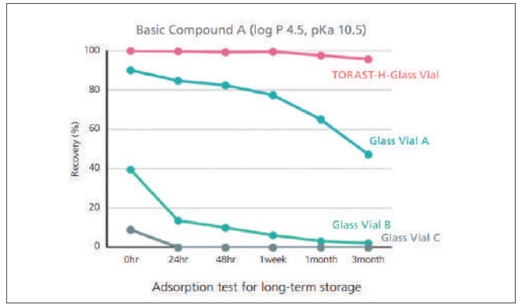
TORAST-H Glass Vial

benefits

- Minimized adsorption of bases, acids and neutrals
- Two sizes are available (1.5 mL and 150 µL)
- Superior Quality Control (Comes with quality certicates for lot management)

Low Adsorption 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.



Adsorption test for long-term 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.

*The area value at 0 h of polypropylene (PP) vial (control) was regarded as 100%, and each area value was compared. The recovery rate of PP vials after 3 months was 89%, which was lower than that of TORAST-H glass vials.

Product Name	Specification	Cap	Slit	Volume	Qty.	P/N
TORAST-H Glass Vial (PTFE, Including vials, caps and septum, cetification of inspection)	glass, clear, label and filling lines (wide open 9-425)	Screw, Black	–	1.5 mL	100 pcs. / pack	370-04300-01
			+			370-04300-02
	glass, amber, label and filling lines (wide open 9-425)		–			370-04300-03
			+			370-04300-04
	glass, clear, label and filling lines (wide open 9-425)		–	150 µL		370-04301-01
			+			370-04301-02
	glass, amber, label and filling lines (wide open 9-425)		–			370-04301-03
			+			370-04301-04
Screw Cap for TORAST-H Glass Vial	PTFE / silicone septum (wide open 9-425)		–	–		370-04310-01
			+			370-04310-02

index

Original Low Adsorption Polypropylene Vial

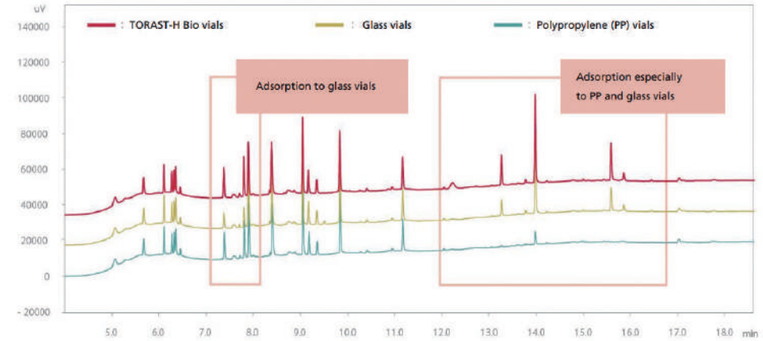
TORAST-H Bio Vial

benefits

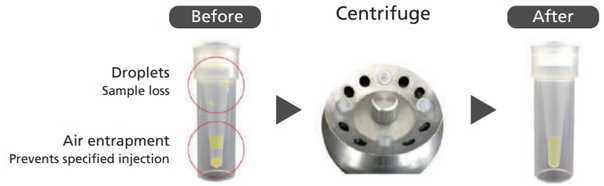
- Easy-to-use, user-friendly design

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 Name	Specification	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..

index

TORAST-H Series

This is a series of high-end consumables that Shimadzu GLC has evaluated and verified using the latest technology.

Low Adsorption Tip for Micro Pipette/Plate

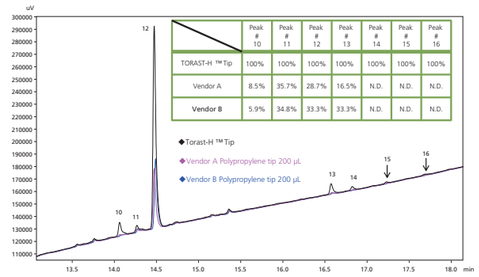
TORAST-H Tip/TORAST-H 96well 500RU

benefits

- Superior Quality Control

Adsorption Test Using Trypsin Digestion Products of Myoglobin (approx. 8.5 pmol/µL)

Since most of the micropipette tips used for sample preparation are made of PP, it is necessary to suppress the hydrophobic adsorption to the tip at low concentration samples. To address this issue, Shimadzu GLC has developed a low adsorption capacity (TORAST-H Tip 200) tip that chemically bonds a nonionic hydrophilic group to the surface of the 200 µL PP tip.



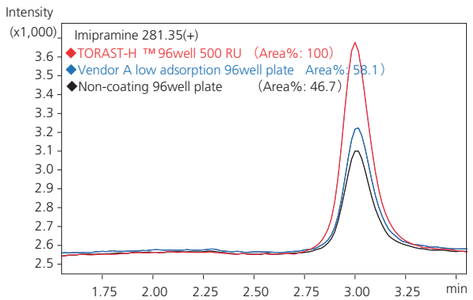
	Peak # 10	Peak # 11	Peak # 12	Peak # 13	Peak # 14	Peak # 15	Peak # 16
TORAST-H ™Tip	100%	100%	100%	100%	100%	100%	100%
Vendor A	8.5%	35.7%	28.7%	16.5%	N.D.	N.D.	N.D.
Vendor B	5.9%	34.8%	33.3%	33.3%	N.D.	N.D.	N.D.

Highly hydrophobic peptides with a retention time of approximately 14 ~ 18 minutes were adsorbed onto commercially available PP tips, but significant suppression of adsorption was confirmed with the TORAST-H Tip.

Realization of High Reproducibility by using a Low-adsorption Well Plate

The low adsorption 96 well plate has a non-ionic hydrophilic group fixed on the inside surface of the well.

One of the most hydrophobic basic compounds is imipramine. Here, the adsorption result of imipramine to the 96 well plate by LC/MS was shown. The TORAST-H 96 well 500 RU showed a remarkable adsorption suppression effect compared with the non-coated 96 well plate and the low-adsorption 96 well plate of other companies. The TORAST-H 96 well 500 RU showed high reproducibility as a result of evaluating the dispersion of adsorption compared with the non-coated well plate.



	%RSD
TORAST-H 96well 500 RU	CV:5.3%
Non-coated 96well plate	CV:18.7%

Quantification of samples and reagents before analysis: Analytical Balances

AP W-AD Series with Automatic Door

benefits

- Fast response and high stability
- Smart Auto Door Improves Measurement Workability
- LabSolutions Balance for Data Reliability

The standard movable windshield inner plate and ionizer improve stability and responsiveness.

The smaller the volume in the weighing chamber, the less the effect of convection and air flow. The model with a minimum display of 0.01 mg is equipped with a movable windshield internal plate as standard. By moving up and down according to various containers and samples, anyone can carry out stable weighing work. In addition, this equipment is equipped with a windless type ionizer STABLO-AP that can eliminate the effects of static electricity in the weighing chamber as standard equipment. Ionizer quickly removes static electricity, improving reproducibility and work efficiency.

Auto Door with Touchless Sensor and Automatic Learning Function

Auto door with touchless sensor improves user's work efficiency. This is because it is touchless and allows non-contact weighing work without touching the operation keys, allowing the user to work while holding the spatula in his hand. In addition, the AP series door with automatic learning function allows you to freely set the opening and closing range of all doors to minimize the effects of outside air. The multi-function mode allows you to assign a total of four individual functions depending on how long you hold your hand over the sensors. The main unit need not be touched, making it ideal for working with toxic substances, and gloves can be worn without interfering with accurate operation.

Complies with ER / ES Related Regulations

Many analytical instruments can prevent data tampering and store analysis conditions and operation history in order to ensure the reliability of the data. LabSolutions Balance is a breakthrough software that solves this challenge for electronic balances.

>>Balance Technical Information Movies

AP W-AD series Specification Overview

Model	AP225W-AD	AP135W-AD	AP225WD-AD	AP125WD-AD	AP324W-AD	AP224W-AD
P/N	321-76000-13	321-76000-10	321-76000-12	321-76000-11	321-76000-03	321-76000-02
Capacity	220 g	135 g	220 g / 102 g	120 g / 52 g	320 g	220 g
Minimum display	0.01 mg		0.1 mg / 0.01 mg		0.1 mg	
Minimum Weight *1	20 mg*2				200 mg	

*1 Be compliant with USP Chapter 41. This is the tested value by the weight of the balance's capacity of 5 % (or 5 grams' weight).
The minimum weight value is affected by the installation environment, so it is necessary to measure it in the actual environment of use.
*2 Measurement conditions of W-AD series (0.01 mg models only) are as follows.
- Set the adjustable windbreak plate in the lowest position
- With a shield plate configured around the pan

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