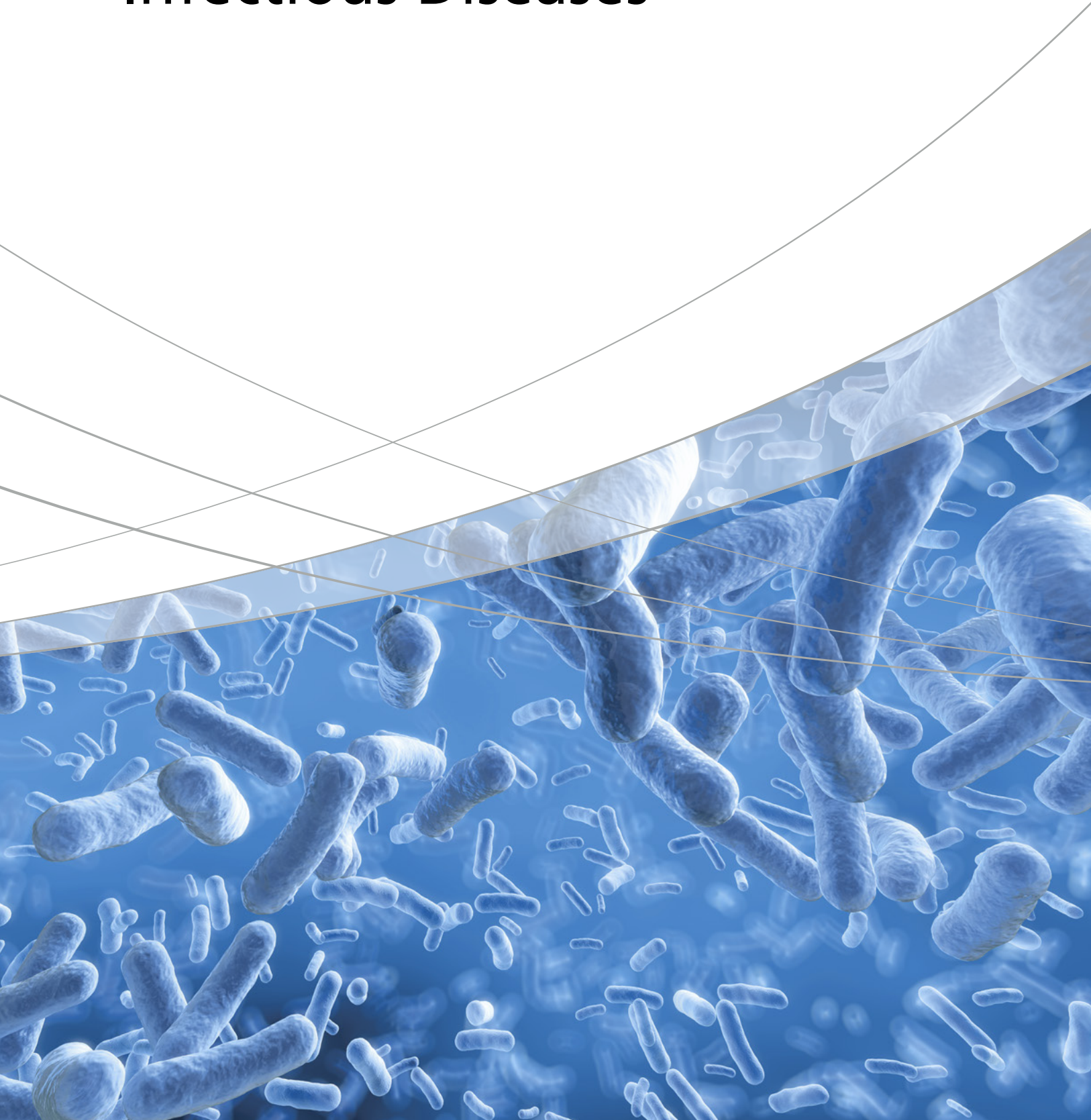![SHIMADZU — Excellence in Science]

Solutions for
# Infectious Diseases

# Infectious Diseases

Infectious diseases are disorders caused by harmful microorganisms (pathogens) such as viruses, bacteria, fungi or parasites. The number of cases for these in the U.S. is led by bacteria with viruses a distant second, with the number of bacterial infectious diseases is on the rise in the last ten years (Figure 1). Given this rise, it is of paramount importance that these infections are investigated and examined. This solution guide introduces a variety of analytical instruments to evaluate such tasks.
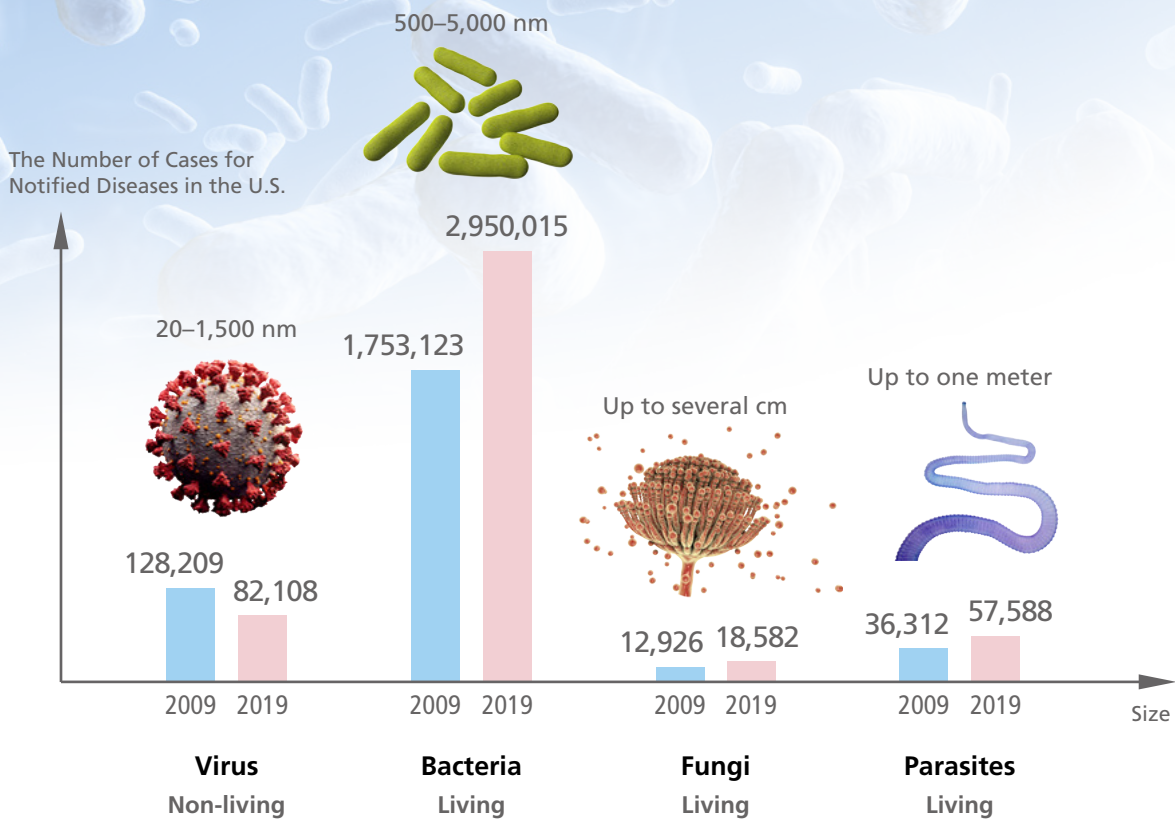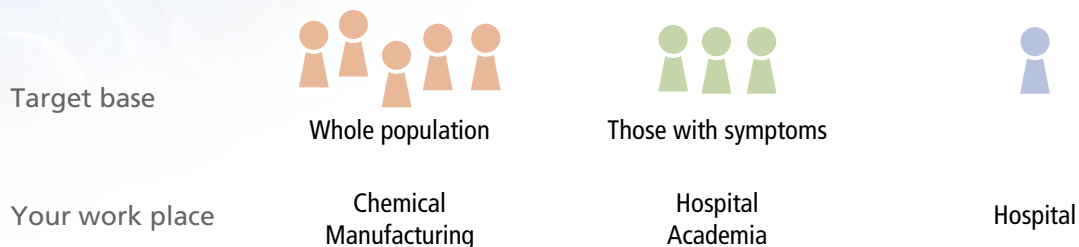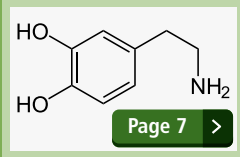
500–5,000 nm

The Number of Cases for
Notified Diseases in the U.S.

2,950,015

20–1,500 nm

1,753,123

Up to several cm

Up to one meter

128,209

82,108

12,926 18,582

36,312 57,588

| 2009 | 2019 | 2009 | 2019 | 2009 | 2019 | 2009 | 2019 | Size |

| **Virus** | **Bacteria** | **Fungi** | **Parasites** |
| Non-living | Living | Living | Living |
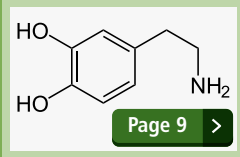
*Fig. 1: Four major types of microorganisms*

In order to exclude the pandemic time, 2019 data was used.
2009: https://www.cdc.gov/mmwr/pdf/wk/mm5853.pdf
2019: https://wonder.cdc.gov/nndss/static/2019/annual/2019-table1.html

This solution guide introduces three analytical instruments: Liquid Chromatography–Mass Spectrometer (LC-MS), Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS), and Gas Chromatography–Mass Spectrometer (GC-MS). A targeted population will be larger at the avoidance phase compared to the testing and care phases. Click on an image to read a specific application.

Target base

Whole population | Those with symptoms

Your work place

Chemical Manufacturing | Hospital Academia | Hospital

**LC, LC-MS**

**MALDI-TOF MS**

**GC, GC-MS**

| Avoidance | Testing | Care |
|---|---|---|
| Vaccine active ingredients / Byproducts | Biomarker search | Drug development |
| Page 4 > | Page 7 > | Page 10 > |
| Vaccine active ingredients | Microorganism identification | |
| Page 5 > | Page 8 > | |
| Alcohol purity testing | Biomarker search | |
| Page 6 > | Page 9 > | |

## Ion-Pair Reversed-Phase LCMS-9030 Mass Spectrometer for Separation and Identification of Oligonucleotides

### Issue

Shorter oligonucleotides (less than 20 bases) can be easily resolved by HPLC, but the separation of those with longer sequences becomes progressively more challenging.

### Solution

Q-TOF LC-MS can be employed to capitalize on its high mass accuracy.

### System Description

**System Configuration**

HPLC System:      Nexera

Column:           Shim-pack Scepter HD-C18-80
                  (2.1 mm I.D. × 100 mm, 1.9 μm)

LC-MS System:     LCMS-9030

Ionization Mode:  ESI (Negative)

**Sample Information**

DNA oligonucleotide standard solution

### Benefits

- High resolution is achieved for the separation of 10 to 60 base oligonucleotides.
- Highly accurate masses are obtained for oligonucleotides.

### Conclusion

10–60 base oligonucleotides were separated and identified.

### Representative Data

*Table 1: The most abundant mass accuracy of oligonucleotides on LCMS-9030*

| Standards | M.W. (Da) | Cal. m/z | Meas. m/z | ppm | Adduction |
|---|---|---|---|---|---|
| 10-mer | 3043 | 1013.1763 | 1013.1773 | 0.99 | $[M-3H]^{3-}$ |
| 15-mer | 4634 | 925.7547 | 925.7557 | 1.08 | $[M-5H]^{5-}$ |
| 20-mer | 6117 | 763.5005 | 763.5020 | 1.96 | $[M-8H]^{8-}$ |
| 25-mer | 7642 | 954.1569 | 954.1585 | 1.68 | $[M-8H]^{8-}$ |
| 30-mer | 9191 | 834.4997 | 834.5014 | 2.04 | $[M-11H]^{11-}$ |
| 40-mer | 12274 | 817.1994 | 817.2013 | 2.33 | $[M-15H]^{15-}$ |
| 50-mer | 15379 | 853.3599 | 853.3615 | 1.87 | $[M-18H]^{18-}$ |
| 60-mer | 18493 | 1026.3331 | 1026.3349 | 1.75 | $[M-18H]^{18-}$ |



*Fig. 2: Separation of 10 to 60 base oligonucleotides*
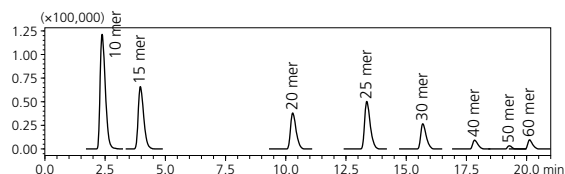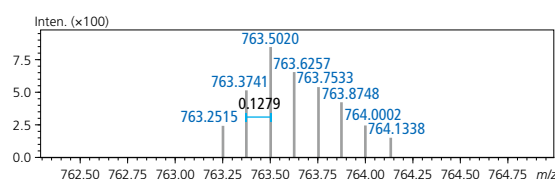


*Fig. 3: High-resolution mass spectrum of 20-base oligonucleotide*

Quadrupole Time-of-Flight Liquid Chromatograph Mass Spectrometer

## LCMS-9030

The LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer is a powerful instrument that integrates the world's fastest and most sensitive quadrupole technology with TOF capabilities for accurate mass measurement.

## Quality Control of Synthetic Peptides Using the MALDI-8030 Dual Polarity Benchtop MALDI-TOF Mass Spectrometer

Application >

### Issue

Quality Control (QC) is critical in providing high-purity products.

### Solution

Negative ionization mode can be used in QC to preserve the integrity of species with labile functional groups.

### System Description

**System Configuration**

MALDI System: MALDI-8030

Matrix Solution: *alpha*cyano-4-hydroxycinnamic acid (CHCA), 5 mg/mL in 1:1 acetonitrile / water, with/without acid (0.1% TFA) depending on the instrument polarity.

**Sample Information**

Synthetic peptides provided by a client

### Benefits

- Peptides with labile functional groups can be analyzed and the detection of the intact species will be viable.
- Mass spectra become cleaner as salt adducts won't be detected.

### Conclusion

Cleaner mass spectra were obtained and QC was conducted.

### Representative Data



Positive mode

| | |
|---|---|
| Peptide D [M+H]+ | *m/z* 2534.982* |
| Loss of 1 SO$_3$ | *m/z* 2455.025* |
| Loss of 2 SO$_3$ | *m/z* 2375.068* |

* calculated

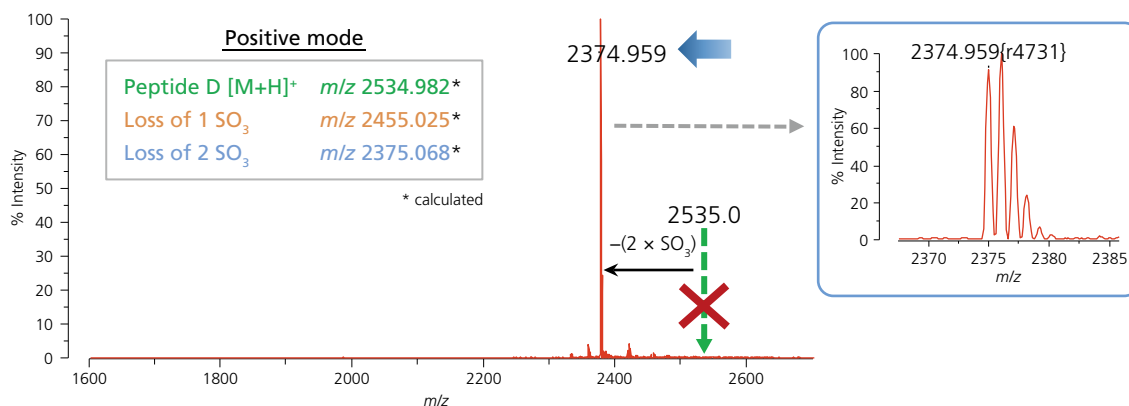2374.959

2535.0
$-(2 \times SO_3)$

2374.959{r4731}

Fig. 4: Positive mode MALDI spectrum of Peptide D: only the species corresponding to the loss in both sulfo groups was detected.

Dual-Polarity Benchtop Linear MALDI-TOF Mass Spectrometer

## MALDI-8030

The MALDI-8030 performance specifications have been extended from those of the MALDI-8020 to cater to 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.

Product >

## Determination of Alcohol Content in Hand Sanitizers by Headspace GC-FID

### Issue

Each country has a minimal purity for ethanol and isopropanol used in hand sanitizers. Sanitizers can contain colorants and thickening agents that can contaminate the GC, however, and render results inaccurate.

### Solution

Headspace GC-FID can be employed instead of a liquid injection system.

### System Description

**System Configuration**

GD-FID:   Nexis GC-2030AF

Headspace Autosampler:   HS-20

Column:   SH-Rxi-624Sil MS (0.32 mm I.D.× 30 m, 1.8 µm)

**Sample Information**

Commercially available hand sanitizers

### Benefits

• Headspace will keep your instrument clean.
• The longer uptime means a higher throughput.
• The cleaner instrument means stable data.

### Conclusion

Hand sanitizers were analyzed with good repeatability and linearity according to EPA 5021.
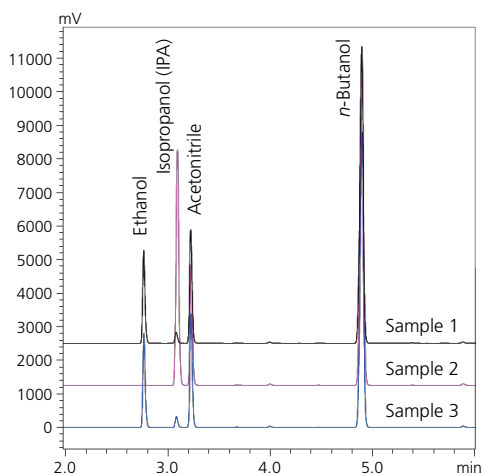
### Representative Data



Fig. 5: Overlaid chromatograms
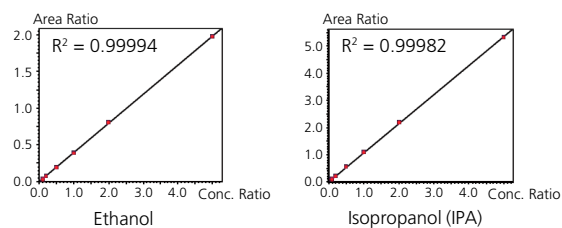of the hand sanitizer samples



Fig. 6: Calibration curves of ethanol and IPA at 0.1 – 5 % (v/v)

$R^2 = 0.99994$

$R^2 = 0.99982$
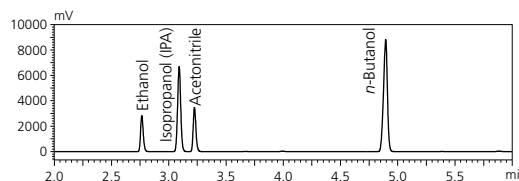


Fig. 7: Separation of the alcohols from the acetonitrile (IS)
both at 2 % (v/v) with a SH-Rxi-624Sil MS column

Gas Chromatograph with Headspace Sampler
## Nexis GC-2030 with HS-20 NX series

The HS-20, developed as the best solution for volatile component analysis, has been improved and introduced as the NX series. The excellent basic performance and user-friendly design provide a powerful solution for scientists in both research and quality control laboratories.

Product >

# Protein Biomarker Quantitation from Human Blood and Plasma Using Novel Collection Technology by LC/MS/MS

## Issue

It takes time and expertise to analyze a wide range of peptides at once.

## Solution

A dedicated system and its accompanying kits (PeptiQuant Comprehensive kit and Biomarker Assessment kit) can be used to analyze a wide range of peptides at once.

## System Description

**System Configuration**

LC-MS System:     LCMS-8060

Column:           Phenomenex Aeris C18
                  (2.1 mm I.D. × 150 mm, 1.7 μm)

**Sample Information**

Dried plasma spots and dried blood spots

## Benefits

Matrix effects can be reduced by using dried plasma spots as a sample compared to dried blood spots.

## Conclusion

27 peptides were monitored from dried blood/plasma spots.

## Representative Data

Peptide TGLQEVEVK
Protein Haptoglobin
501.80 > 246.20(+) CE: −22.6

Peptide FPEVDVLTK
Protein Apolipoprotein C-I
528.30 > 908.50(+) CE: −23.5

Peptide ATVVYQGER
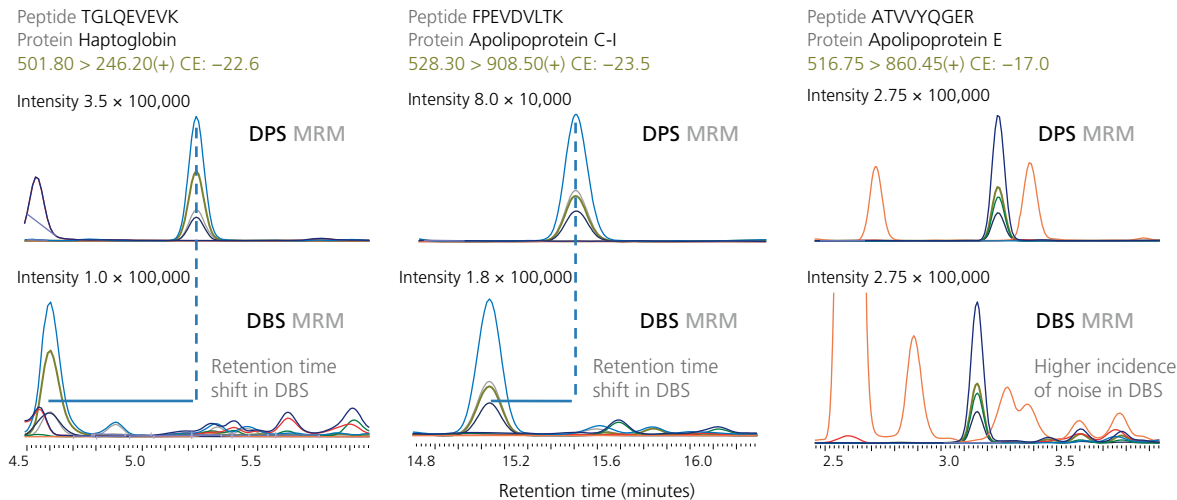Protein Apolipoprotein E
516.75 > 860.45(+) CE: −17.0



*Fig. 8: MRM chromatograms for three peptides; haptoglobin, apolipoprotein C-I and apolipoprotein E. The upper MRM chromatograms correspond to DPS extracts; the lower MRM chromatograms from DBS extracts highlight retention time shifts and a higher incidence of noise in the MRM channel.*

Liquid Chromatograph Mass Spectrometer
## LCMS-8060NX

The LCMS-8060NX is a triple quadrupole mass spectrometer with world-class sensitivity and detection speeds. It boasts increased robustness and ease of use as well as Analytical Intelligence to maximize your laboratory's output.

## Application of MALDI-TOF MS for Discrimination of Species Processing Highly Conserved Ribosomal RNA Gene Sequences

**Application** >

### Issue

Identification can be difficult due to a high homology rate of 16S rRNA gene sequences.

### Solution

A dedicated system and its accompanying database can be used to identify microorganisms.

### System Description

**System Configuration**

MALDI System: AXIMA Microorganism Identification System

**Sample Information**

Harvested microorganisms

### Benefits

A dedicated database allows you to compare an unknown mass spectrum to a reference for a more confident identification.

### Conclusion

*E. Coli* species were identified.

### Representative Data

Table 2: Agreement rate of rRNA gene sequences
(From Muroi et al. (2011) with permission from Pharmaceutical Society of Japan)

| Species | Strains | No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | NBRC 12734 | 1 | | | | | | | | | | | |
| | NBRC 3972 | 2 | 99.1 | | | | | | | | | | |
| | NBRC 12713 | 3 | 99.7 | 99.3 | | | | | | | | | |
| | NBRC 13168 | 4 | 99.4 | 99.5 | 99.3 | | | | | | | | |
| | NBRC 13891 | 5 | 99.9 | 99.3 | 99.8 | 99.5 | | | | | | | |
| | NBRC 13893 | 6 | 99.7 | 99.3 | 100.0 | 99.3 | 99.8 | | | | | | |
| | NBRC 3301 | 7 | 99.7 | 99.0 | 99.7 | 99.2 | 99.5 | 99.7 | | | | | |
| | NBRC 102203 | 8 | 99.5 | 99.0 | 99.5 | 99.2 | 99.7 | 99.5 | 99.3 | | | | |
| | NBRC 14237 | 9 | 99.7 | 99.2 | 99.7 | 99.3 | 99.8 | 99.7 | 99.4 | 99.6 | | | |
| *E. fergusonii* | NBRC 102419 | 10 | 99.6 | 98.9 | 99.5 | 99.3 | 99.6 | 99.5 | 99.7 | 99.3 | 99.5 | | |
| *E. hermanii* | NBRC 105704 | 11 | 97.4 | 97.4 | 97.3 | 97.5 | 97.5 | 97.3 | 97.1 | 97.2 | 97.4 | 97.1 | |
| *E. blattae* | NBRC 105725 | 12 | 95.9 | 96.0 | 96.0 | 96.0 | 96.0 | 96.0 | 95.8 | 95.8 | 95.9 | 96.0 | 95.8 |

MALDI-TOF MS System

## AXIMA Microorganism Identification System

The AXIMA Microorganism Identification System presents a new solution for the identification of microorganisms in the pharmaceutical, food, science, energy, environment, clinical and health fields.

**Product** >

## Detection of Tuberculosis Biomarkers by Multidimensional GC-MS

**Application** >

### Issue

Tuberculosis infects one third of the world population, and biomarkers are needed to aid with the diagnosis. Matrix can be complex and pose an analytical issue.

### Solution

Heart-cut GC-MS was used to mitigate adverse effects from matrix interferences.

### System Description

**System Configuration**
GC-MS System:       Multi-Dimensional GC-MS System

**Sample Information**
Sputum

### Benefits

Sample matrix can be eliminated for an easier detection of biomarkers.

### Conclusion

20 potential biomarker compounds were found.

### Representative Data



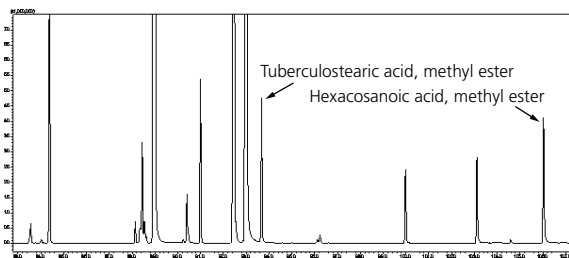Tuberculostearic acid, methyl ester
Hexacosanoic acid, methyl ester

Fig. 9: The 2nd dimension GC-MS chromatogram of the sputum sample. The clean chromatogram allows accurate quantification of the 20 biomarkers.
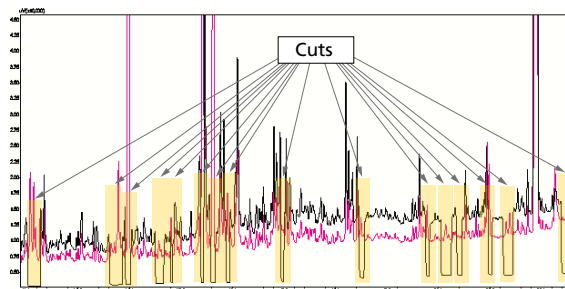


Cuts

Fig. 10: The 1st dimension FID chromatogram of the sputum sample in standby mode (Pink) and in cut mode with 16 cut positions for 20 target compounds (Black).

Multi-Dimensional GC/GCMS System
# MDGC/GCMS-2010 series

The complete separation and quantification of specific compounds can be difficult in samples that contain many compounds, such as petroleum products and fragrance products. Multi-Dimensional GC (MDGC) uses columns with different separation characteristics to achieve highly accurate separation of target components from such complex matrices.

**Product** >

## Simultaneous Analysis of Remdesivir and Metabolites in Human Plasma Using Fully Automated Sample Preparation LC/MS/MS System

**Application** >

### Issue

Manual sample extraction can be labor-intensive.

### Solution

A sample preparation system can be fully automated.

### System Description

**System Configuration**

| | |
|---|---|
| UHPLC: | Nexera X2 |
| Column: | Shim-pack Scepter C18-120 (2.1 mm I.D. × 50 mm, 1.9 µm) |
| LC-MS System: | LCMS-8060 |
| Ionization Mode: | ESI (Positive) |

**Sample Information**

Commercially available human plasma

### Benefits

- Sample extraction can be automated.
- Automation reduces method variability, the chance of sample mix-ups and the risk of exposure to samples.

### Conclusion

Automation not only allowed for walk-away times but yielded good accuracy and reproducibility.
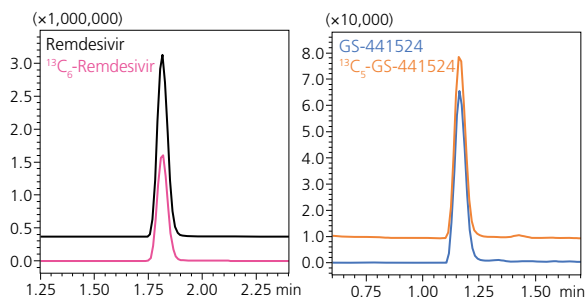
### Representative Data



*Fig. 11: MS Chromatograms of Remdesivir, [U-Ring-$^{13}C_6$]-Remdesivir (Left) and GS-441524, [$^{13}C_5$]-GS-441524 (Right)*

*Table 3: Repeatability of Remdesivir and GS-441524 in plasma*

| Compounds | QC Sample | Spiked Conc. (ng/mL) | Intra-Assay ($n = 6$) | | |
|---|---|---|---|---|---|
| | | | Average Conc. (ng/mL) | Precision %RSD | Accuracy % |
| Remdesivir | LLOQ | 100 | 90.5 | 2.0 | 91 |
| | Low | 750 | 797 | 1.7 | 106 |
| | Medium | 1000 | 1045 | 0.9 | 105 |
| | High | 3750 | 3393 | 2.0 | 91 |
| GS-441524 | LLOQ | 5 | 4.51 | 3.1 | 90 |
| | Low | 37.5 | 33.2 | 2.5 | 89 |
| | Medium | 50 | 45.2 | 2.3 | 90 |
| | High | 187.5 | 171.7 | 3.6 | 92 |



Fully Automated Sample Preparation Module for LC-MS

# CLAM-2040

Operational improvements in LC-MS systems have made LC/MS an advantageous technique for clinical research, thanks to its specificity, its accuracy and its capability to analyze several targets simultaneously. The CLAM-2040 is an online automated sample preparation module that brings LC-MS smoothly into your laboratory.

**Product** >

## JP- and EP-Compliant Analysis of Impurities of COVID-19 Drug Dexamethasone

### Issue

Pharmaceutical companies need to control impurities strictly from a safety perspective.

### Solution

LC-MS can be used to monitor impurities.

### System Description

**System Configuration**

HPLC System:    Nexera lite

Column:         Shim-pack VP-phenyl
                (4.6 mm I.D. × 250 mm, 5 µm)

Detection:      UV 254 nm

**Sample Information**

Dexamethasone standard solution

### Benefits

System suitability tests are satisfied for both Japanese and European Pharmacopeia using the triple quad LCMS-8060NX.

### Conclusion

Impurity testing was carried out according to Japanese and European Pharmacopeia.

### Representative Data
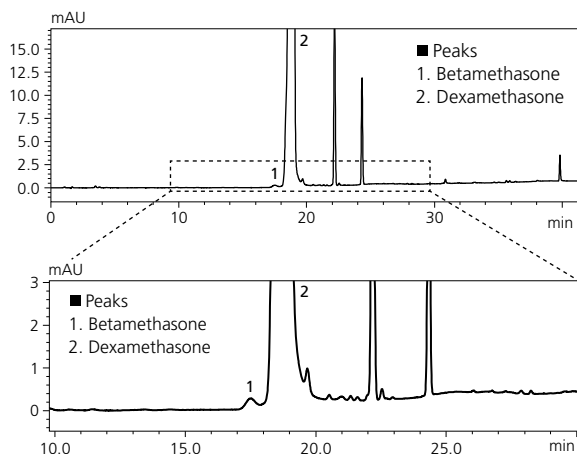


*Fig. 12: Chromatogram of Reference Solution (a) (European Pharmacopoeia)*
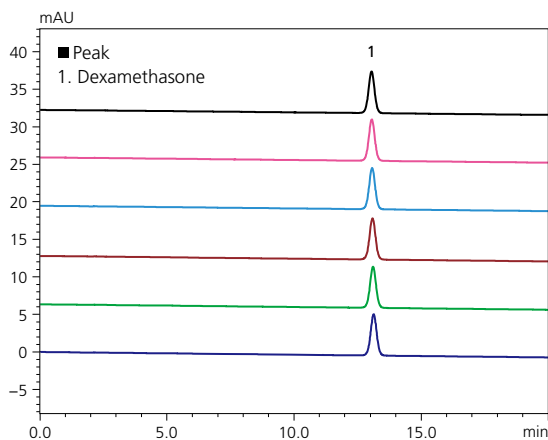


*Fig. 13: Comparison of Chromatograms in 6 Repeated Tests with Standard Solution (5.94 mg/L) (Japanese Pharmacopoeia)*

Ultra High Performance Liquid Chromatograph

## Nexera series

The Nexera series is a family of HPLC systems that marries AI and IoT enhancements to set new industry standards. The Nexera lite is a conventional HPLC model with excellent basic functions and many options for expansion. Cutting-edge AI functions increase the reliability of established analysis workflows and adaptable to any application system.

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