Mass Spectrometry – A Powerful tool for metabolomics

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Shimadzu [Asia-Pacific] Pte Ltd
Biomarkers Identification and Validation

Basic Research
Biobanking
New Technologies

Translational Research

Clinical Methods

Discovery
Qualification
Verification
Validation

Number of analytes
Number of samples
Mass Spectrometry – For Metabolomics

- GCMS-QP2010 Ultra
- GCMS-TQ 8040
- LCMS-8060
- Nexera UC- Online SFE/SFC
- iDPlus-Performance Bacterial Identification
- MALDI -7090 High Resolution TOF
- iMScope-Trio –Mass imaging
Triple Quadrupole MS/MS

1) Product ion scan

Ion source → Quadrupole (Q1) → Collision cell → Quadrupole (Q2) → Quadrupole (Q3) → detector
MRM - Multi Reaction Monitoring

SIM (single analysis)

10 ppb

High sensitivity but high background

MRM (MS/MS analysis)

10 ppb

Eliminates background for trace-level quantitation with high S/N
GCMS- Gold Standard for Metabolomics
Metabolomics Research Using GC-MS/MS

**Discovery phase**
- Scan measurement (non-targeted analysis)
- Detect marker candidates and identify compounds

**Validation phase**
- MRM measurement (GC-MS/MS) (target analysis)
- Quantitate marker candidates with higher accuracy

**Metabolomics Research**

- MRM measurement (wide target analysis)
GC/MS Metabolite Database

Smart Metabolites Database
Database Software for GC/MS and GC-MS/MS Analysis of Metabolites

Registered Compounds Derivatives Measurement Mode Number

Organic acids, fatty acids, amino acids, sugars, etc.
TMS Scan 428 MRM 193

Fatty acids Methylation Scan 50 MRM 50

Amino acids EZ:faastTM Scan 33
Easy Work Flow

AART function for Automatic Adjustment of Retention Indices with just one injection

Select components for measurement from the database.

Method is created Automatically

Start acquisition.
Three Smart functions improve analytical productivity in your laboratory.

1. **Smart Productivity**
   - Analysis of 400 pesticides that used to require 2 or 3 methods, can now be accomplished in a single acquisition method by the new firmware protocol.

2. **Smart Operation**
   - **Smart MRM** technology creates optimal MRM methods automatically. The “MRM Optimization Tool“ automates best MRM transitions for new compounds.

3. **Smart Performance**
   - ASSP achieves high sensitivity at scan speeds of 20,000 u/second. Fastest MRM 800trans/sec. Single GC/MS mode with the maximum possible sensitivity and repeatability.
Advantages of GCMSMS

Comparison of Reproducibility for Measuring Metabolites in Human Blood Serum Using MRM and Scan Modes

<table>
<thead>
<tr>
<th>Compound name</th>
<th>%RSD (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxyisovaleric acid-2TMS</td>
<td>3.99</td>
</tr>
<tr>
<td>Homocysteine-3TMS</td>
<td>5.04</td>
</tr>
<tr>
<td>Aconitic acid-3TMS</td>
<td>5.98</td>
</tr>
<tr>
<td>Kynurenine-3TMS</td>
<td>6.48</td>
</tr>
</tbody>
</table>

MRM is able to eliminate the effects of interfering substances so that it can measure trace components accurately.

193 types of trimethylsilylated metabolites
50 types of fatty acid methyl esters (compatible with EI/PCI ionization)
Advantage of GCMSMS

Scan/MRM data

Scan (Non-targeted)

MRM (Targeted)

Measurement of Scan/MRM mode (SmartMRM)
Scan section: Comprehensive search of metabolites
MRM section: Accurate quantitation of target compounds

Discovery

Validation

Ex. TCA Cycle
Metabolites in urine using Scan/MRM

Scan

Semi-quantitation

Malic Acid-3TMS

Adipic acid-2TMS (MRM)

Glutaric acid-2TMS (MRM)

Measured

Library

Identification

Measurement spectrum

Library spectrum
Metabolomics Solutions
– Measurements to Pathway Analyses

Scan

MRM

Measure by GC-MS(/MS)

Enter PubChem ID

Identify compounds

GC/MS Metabolite Database Ver. 2

Output data

From PubChem, link to KEGG

Analyze pathway data

Handbook

Multivariate analysis
(Detect marker candidates)

Scan

GC/MS Metabolite Database Ver. 2

CID 161166

Handbook

Multivariate analysis
(Detect marker candidates)
Conventional Metabolomics

**LC-MS**
- Peptide, Coenzyme
- Nucleotide, Lipids
- Steroid, Vitamin
- Nucleoside, Sugar phosphate
- Sugar, Amino acids
- Organic acids, Fatty acids

**GC-MS**
- Volatility
- Terpene, Hydrocarbo, Ester, Ketone, Alcohol

**M. W.**
- Large
- Small

Non Volatility
LCMSMS – Readymade Solutions

LC/MS/MS Method Package for Lipid Mediators Ver. 2

LC/MS/MS Method Package for Cell Culture Profiling
For LabSolutions Version 5

LC/MS/MS MRM Library for Metabolic Enzymes in Yeast
For LabSolutions Ver. 5
LCMSMS - Readymade Solutions

LC/MS/MS Method Package for Primary Metabolites Ver. 2
For LabSolutions Ver. 5
Metabolites for major pathways in single method

### Ion pairing method (55 compounds)

- **Glycolysis**
  - 2,3-Bisphosphoglyceric acid
  - 3-Phosphoglyceric acid
  - Dihydroxyacetone phosphate
  - Fructose 1,6-bisphosphate
  - Glucose 1-phosphate
  - Glucose 6-phosphate
  - Glyceraldehyde 3-phosphate

- **Pentose phosphate pathway**
  - 2,3-Bisphosphoglyceric acid
  - 3-Phosphoglyceric acid
  - Dihydroxyacetone phosphate
  - Fructose 1,6-bisphosphate
  - Glucose 1-phosphate
  - Glucose 6-phosphate
  - Glyceraldehyde 3-phosphate

- **TCA cycle**
  - 2,3-Bisphosphoglyceric acid
  - 3-Phosphoglyceric acid
  - Dihydroxyacetone phosphate
  - Fructose 1,6-bisphosphate
  - Glucose 1-phosphate
  - Glucose 6-phosphate
  - Glyceraldehyde 3-phosphate

- **Amino acids**
  - Alanine
  - Arginine
  - Asparagine
  - Aspartic acid
  - Cysteine
  - Glutamic acid
  - Glutamine
  - Glycine
  - Histidine
  - Lysine
  - Methionine
  - Phenylalanine
  - Serine
  - Threonine
  - Tryptophan
  - Tyrosine

- **Nucleotides**
  - Adenosine 3',5'-cyclic monophosphate
  - Adenosine diphosphate
  - Adenosine monophosphate
  - Adenosine triphosphate

- **Coenzymes**
  - FAD
  - FMN
  - NAD
  - NADH
  - NADP
  - NADPH

### Non-ion pairing method (97 compounds)

- **Glycolysis**
  - 2,3-Bisphosphoglyceric acid
  - 3-Phosphoglyceric acid
  - Dihydroxyacetone phosphate
  - Fructose 1,6-bisphosphate
  - Glucose 1-phosphate
  - Glucose 6-phosphate
  - Glyceraldehyde 3-phosphate

- **Pentose phosphate pathway**
  - 2,3-Bisphosphoglyceric acid
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- **TCA cycle**
  - 2,3-Bisphosphoglyceric acid
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  - Dihydroxyacetone phosphate
  - Fructose 1,6-bisphosphate
  - Glucose 1-phosphate
  - Glucose 6-phosphate
  - Glyceraldehyde 3-phosphate

- **Amino acids**
  - Alanine
  - Arginine
  - Asparagine
  - Aspartic acid
  - Cysteine
  - Glutamic acid
  - Glutamine
  - Glycine
  - Histidine
  - Lysine
  - Methionine
  - Phenylalanine
  - Serine
  - Threonine
  - Tryptophan
  - Tyrosine

- **Nucleotides**
  - Adenosine 3',5'-cyclic monophosphate
  - Adenosine diphosphate
  - Adenosine monophosphate
  - Adenosine triphosphate

- **Coenzymes**
  - FAD
  - FMN
  - NAD
  - NADH
  - NADP
  - NADPH

- **Organic acids**
  - Lactic acid
  - Pyruvic acid

- **Standards**
  - 2-Morpholinoethanesulfonic acid
  - Methionine sulfone

Ion pairing method contains metabolites in the central carbon metabolic pathway as target compounds and some amino acids and organic acids are added as target compounds in non-ion pairing method.
# Method Package for Primary Metabolites

<table>
<thead>
<tr>
<th></th>
<th>Ion pairing method (55 compounds)</th>
<th>Non-ion pairing method (97 compounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target compounds</strong></td>
<td>✓ Glycolysis, TCA cycle (CoA), Pentose phosphate pathway, Amino acids, Nucleotides</td>
<td>Amino acids, TCA cycle (organic acid), Bases, Nucleosides, Nucleotides, Transsulfuration pathway, Methylation cycle</td>
</tr>
<tr>
<td><strong>LC conditions</strong></td>
<td>✓ Usage of ion pairing reagent</td>
<td>✓ Ion pairing reagent is not used</td>
</tr>
<tr>
<td></td>
<td>✓ Separation by ODS column</td>
<td>✓ Separation by pentafluorophenylpropyl (PFPP) column</td>
</tr>
<tr>
<td><strong>Targeted Application</strong></td>
<td>✓ Medical researcher in pharmaceutical company and academia</td>
<td>✓ Medical researcher in pharma and academia (ion pairing reagent is not allowed to use)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>✓ Quality assessment by quantifying amino acids, organic acids etc.</td>
</tr>
</tbody>
</table>
An aliquot of 57 STDs Mix was injected at a volume of 3 µL and separated using an ion-pairing chromatography.

Column: Mastro C18 (2.0 X 150 mm, 3 mm)
Mobile phase: A: 15 mmol/L Acetate, 10 mmol/L Tributylamine - Water
B: Methanol
Injection vol.: 3 µL (100 nM 55 STD Mix)
Dwell Time: 10 ms
Ionization mode: ESI negative
97 Standards Mix: Non-ion pair method

Standards mix at 500 nM was analyzed at a injection volume of 3 µL.

Metabolites related to transsulfuration pathway and methylation cycle were added as new targets.

Column: Discovery HS F5-3 (2.1 X 150 mm, 3 mm)
Mobile phase: A: 0.1% formic acid – Water, B: 0.1% formic acid – Acetonitrile
Injection vol.: 3 mL (1 mM 97 STD Mix)
Dwell Time: 5 ms, Ionization mode: ESI/positive/negative

Pentafluorophenylpropyl functional group
Shimadzu series of LCMSMS

GLOBAL LAUNCH

LCMS-8030
First mass spectrometry company to achieve a scan speed of 15,000u/sec and polarity switching time of 15msec

2010 AUGUST

LCMS-8040
Increased sensitivity by a factor of 5 compared to the LCMS-8030

2012 MAY

LCMS-8050
First mass spectrometry company to achieve 5msec polarity switching time and a scan time of 30,000u/sec. Increased sensitivity by 30 times compared to LCMS-8030

2013 AUGUST

LCMS-8060
A new vision in sensitivity. It simply changes everything. Increased sensitivity by 90 times compared to LCMS-8030

2015 ASMS 2015 MAY

LCMS-8030 LCMS-8040 LCMS-8050 LCMS-8060
Frost & Sullivan, which is one of the world's leading business intelligence giants, has highlighted in its prestigious white paper on best industry practices that:

• Shimadzu has established a strong presence in the APAC mass spectrometry market leveraging on its strengths in product innovation, high value-added and quality mass spectrometers, and excellent promotional activities.

• Shimadzu is working towards increasing its production capacity, thus ensuring the stable and reliable supply of products for years to come.

• Shimadzu's outstanding cost structure will further fortify its position as a leading participant in the MS market.

• Frost & Sullivan considered 2 key factors while deciding on the Company of the Year Award: Shimadzu's Visionary Innovation and Performance (This included criteria like Addressing Unmet Needs, Visionary Scenarios Through Mega Trends, Implementation Best Practices, Blue Ocean Strategy and Financial Performance) and Customer Impact (This included criteria like Price/Performance Value, Customer Purchase Experience, Customer Ownership Experience, Customer Service Experience and Brand Equity).
LCMS-8060: Changes Everything

1. Highest Sensitivity
2. Ultra Fast Technologies
3. Unsurpassed Robustness

Increased ion production
Changes to the desolvation line capillary have increased ion production by a factor of >3

UF Qarray
Redesigned to deliver a meaningful impact on ion focusing capability and higher sensitivity

Enhanced vacuum
New vacuum designed to increase ion transmission
MRM chromatograms of rat’s kidney

- Extraction corresponds to 5 mg of kidney tissue followed by 100 times dilution
- Sample amounts being measured: 3 μg of tissue

1. Quickly freezing a rat tissue by LN2
2. Weighing the frozen tissue and homogenizing
3. Adding 70% MeCN which is 10 times of sample volume
4. Collecting supernatants after centrifuging
5. Collecting supernatants by MeOH / CH3Cl
6. Filtration
7. Drying it up by SpeedVac
8. Reconstitution
9. Dilution and injection onto LCMS-8060

Column: Discovery HS F5 (3.0 μm, 2.1 X 150 mm)
Inj. Vol.: 3 μL
MPs: 
A: 0.1% Formic acid-Water
B: 0.1% Formic acid-Acetonitrile
Metabolites Profiling in Rat Kidney

- Area ratio against ITSD
- 85 compounds are detected among 97 compounds
Metabolite Variation by Drug

- The level of ophthalmic acid, glutathione and 5-Glu-Cys were changed by the administration of drug A

Graph has shown average of area ratio (n=4)
Full Line of Shimadzu
Ultra Fast Mass Spectrometers

offers

High sensitivity and Enhanced selectivity
High-Speed Performance

GCMS-QP2010 Ultra
GCMS-TQ8040
LCMS-8060
GCMS-QP2010 SE
LCMS-8030
LCMS-8040
LCMS-8050
LCMS-2020

Full Line of Shimadzu
Ultra Fast Mass Spectrometers

offers

High sensitivity and Enhanced selectivity
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GCMS-TQ8040
LCMS-8060
GCMS-QP2010 SE
LCMS-8030
LCMS-8040
LCMS-8050
LCMS-2020
Conventional Metabolomics

Supercritical Fluid Chromatography [SFC-MS]

M. W. | Large

V. W. | Small

V. W. | Volatility

Non Volatility

GC-MS

Terpene

Nucleoside, Sugar phosphate

Peptide, Coenzyme, Nucleotide, Lipids

Steroid, Vitamin

N. W.

LC-MS
Online SFE/SFC/MS system

- **CO₂ Cylinder**
- **CO₂ pump (Pump A) LC-30AD₅F**
- **Modifier pump (Pump B) LC-20ADₐ** etc.
- **Extraction vessel**
- **Valve**
- **Valve**
- **SFE Unit**
- **Make up solvent**
- **Make up solvent pump (Pump C) LC-20ADₓᵣ**
- **Splitter**
- **Column oven CTO-20AC etc.**
- **BPR-B (Back Pressure Regulator) SFC-30A**
- **BPR-A (Back Pressure regulator) SFC-30A**
- **Injector**
- **Drain**
- **Column**
- **MS**
- **LCMS-TQ (8030/8040/8050/8080)**
- **Injector drain 7% 93%**
Advantage of SFC

- high sensitivity
- fast and high separation
- suitable for separation of hydrophobic compounds
- possible to change the polarity using modifier

1. Diazinon  8. Aramite
3. Tolclofos-methyl  10. Acephate
4. Lenacil  11. Aminocarb
5. Mepronil  12. Cyazofamid
          15. Imidacloprid
<table>
<thead>
<tr>
<th>Target</th>
<th>Enabling Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell</td>
<td>MS Microscope</td>
</tr>
<tr>
<td>Tissue/Organ</td>
<td>MALDI-TOF MS Imaging</td>
</tr>
<tr>
<td>Small animals</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>PET</td>
</tr>
<tr>
<td>Rat</td>
<td>CT</td>
</tr>
<tr>
<td></td>
<td>Optical Imaging</td>
</tr>
<tr>
<td>Human</td>
<td>fNIRS</td>
</tr>
<tr>
<td>Brain</td>
<td>LABNIRS</td>
</tr>
<tr>
<td>Whole body</td>
<td>Clinical PET/CT</td>
</tr>
</tbody>
</table>

- **In vitro**
- **In vivo**
Imaging – Current challenges

Conventional optical microscope

iMScope- MS microscope

Image obtained with optical microscope

Simultaneous molecular identification by MS under microscope observation.

Images obtained with MS microscope

Identification of substances

No information on compounds

Laser radiation on a small spot

Abnormal cell?
iMScope Trio– Imaging Mass Microscope
iMScope – Imaging Mass Microscope

**Diagram Elements:**
- **Optical microscope**
- **Sample**
- **Sample stage**
- **Observation spot**
- **Transmission lamp**
- **Laser**
- **Ion guide**
- **Sample inlet**
- **QP / Ion trap**
- **Ionization point**
- **Detector**
- **Time of flight mass spectrometer**
iMScope- How it works

Nd:YAG Laser (355nm) → Quadrupole Ion Trap → Time-of-Flight Mass Analyzer with Reflectron

Optical microscope

Reflected Light Illuminator

Sample

Transmitted Light Illuminator

RF Ion Guide

Laser Beam

Sample

Optical observation

Analyzing Position

Observing Position

3-Dimensional Automated Stage

Interface
Direct MS measurement of tissue slice
- visualize a local distribution of compounds
High spatial resolution (5um)
- observe compounds in a single cell
Atmospheric pressure ionization
- Suitable for living cell
MS^n measurement
- realize structural analysis of unknown compounds
Powerful Software

(1) Optical Microscope Image

(2) Assignment of analyzing spot / area

(3) Setting of mass analyses parameters

(4)-1 Real time mass spectrum

(4)-2 MS Image
Imaging Viewer software
iMScope – Drug Delivery System

Targeting of drug to tumor

Targeting of drug to cancer cells

Controlled release of drug

DDS

15min

1hr

24hrs

Tumor

Taxol in tumor

Taxol-Micelle in tumor

0%

100%
The peak $m/z$ 616 can be identified as Heme b based on MS$^n$ analysis. Heme b concentration is found to be low due to ischemic conditions at cancer area.
iMScope – Biological Tissue Slice

Visualizing the distribution of lipid isomers in the mouse retina to understand the structure of the lipid layers.

- m/z772.5 PC(16:0 16:0)+K
- m/z798.5 PC(16:0 18:1)+K
- m/z806.5 PC(16:0 22:6)+H
- m/z872.4 PC(18:0 22:6)+K

Inside of retina

Outside of retina

Superimposed
iMScope – Potential Applications

Medical diagnostics
Distribution of compounds in lesion area
Optical imaging  MS imaging

Drug metabolism
Distribution of drug A (m/z 313) in tissue
Optical imaging  MS imaging
Tissue (control)
Tissue (with drug A)

Agriculture
Distribution of active ingredient in rice
Optical imaging  MS imaging (Distribution of LPC)
New rubber  Old rubber

Chemical material
Distribution of compound B in rubber sheet
New rubber  MS imaging  Old rubber
Summary

- Shimadzu offers wide range of analytical instruments from Research to routine analysis
- In addition to analytical platforms Shimadzu offers readymade solutions to enhance your workflows
- iMScope can be extremely useful in various fields especially in Clinical Metabolomics due to its high spatial resolution.
- Imaging is powerful tool to study the localization of compounds within the cells/Tissues
- Shimadzu is keen to collaborate for your research with its advance instrumentation to ramp up your research