Ionization Methods for JEOL Mass Spectrometers

- A Guidebook -

Different ionization methods for MS applications
- Electron Ionization (EI)
- Chemical Ionization (CI)
- Photo Ionization (PI)
- Field Ionization / Field Desorption (FI/FD)
- Electrospray Ionization (ESI)
- CSI (Cold-Spray Ionization)
- Direct Analysis in Real Time (DART)
- Matrix Assisted Laser Desorption / Ionization (MALDI)
JEOL mass spectrometers support a variety of ionization methods, including ionization combined with chromatography, ionization using direct sample loading, hard ionization, soft ionization, etc.

This guidebook will describe the principles and characteristics of these methods and introduce their applications.

<table>
<thead>
<tr>
<th>JEOL Mass Spectrometers</th>
<th>EI</th>
<th>CI</th>
<th>PI</th>
<th>FI</th>
<th>DEI*1</th>
<th>DCI*1</th>
<th>FD</th>
<th>MALDI</th>
<th>DART</th>
<th>APCI</th>
<th>ESI</th>
<th>CSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS JMS-Q1500GC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC-MS JMS-TQ4000GC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC-TOFMS JMS-T200GC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC-HRMS JMS-800D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas Analysis-MS JMS-MT3010HRGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALDI JMS-S3000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DART™, LC-MS JMS-T100LP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ionization methods marked by deep blue can be combined with chromatography including GC and LC.
*1: DEI and DCI represent EI and CI using a direct loading probe.
*2: The JMS-MT3010HRGA uses EI as a dedicated gas analysis system. It cannot be combined with chromatography.
Soft ionization gives little internal energy to sample molecules, produces fewer fragment ions, and makes it easier to observe molecular ions from ionized sample molecules. Ionization may be accomplished in various ways: by the direct interaction of sample molecules and electrons, through the ion molecular reaction between ionized reagent gas and sample molecules, etc.

On the other hand, hard ionization imparts excess internal energy to sample molecules and produces a large amount of fragment ions. Fragment ions, containing partial structural information of sample molecules, are effective for structural analysis. Electron ionization (EI) is a typical hard ionization method.

JEOL mass spectrometers support a variety of ionization methods. Selecting an ionization method optimum for the sample and applications makes it possible to acquire the best results.

There is no one ionization method to support all applications. You need to select an ionization method best suited for your applications. The table on the left shows the ionization methods you can use with JEOL mass spectrometers. As the table demonstrates, JEOL mass spectrometers support a variety of ionization methods. The figure below shows the molecular weight and sample polarity ideal for each ionization method.

**Topic 1**

**Applicable ranges of ionization methods**

There is no one ionization method to support all applications. You need to select an ionization method best suited for your applications. The table on the left shows the ionization methods you can use with JEOL mass spectrometers. As the table demonstrates, JEOL mass spectrometers support a variety of ionization methods. The figure below shows the molecular weight and sample polarity ideal for each ionization method.

**Topic 2**

**Soft ionization**

Soft ionization gives little internal energy to sample molecules, produces fewer fragment ions, and makes it easier to observe molecular ions from ionized sample molecules. Ionization may be accomplished in various ways: by the direct interaction of sample molecules and electrons, through the ion molecular reaction between ionized reagent gas and sample molecules, etc.

On the other hand, hard ionization imparts excess internal energy to sample molecules and produces a large amount of fragment ions. Fragment ions, containing partial structural information of sample molecules, are effective for structural analysis. Electron ionization (EI) is a typical hard ionization method.

JEOL mass spectrometers support a variety of ionization methods. Selecting an ionization method optimum for the sample and applications makes it possible to acquire the best results.

**Topic 3**

**Ionization for analysis of complex mixed samples**

Complex mixed samples, if ionized as they are, will produce mass spectral data showing peaks from various compounds detected at once, making accurate analysis extremely difficult. For analysis of complex compound mixtures, chromatography is effective in separating compounds.

Different types of chromatography are available: gas chromatography (GC) using the interaction between a vaporized sample solution and a column liquid phase; liquid chromatography (LC) using the interaction between a sample solution and a column solid phase. Ionization methods supported by GC and LC are listed below.

<table>
<thead>
<tr>
<th>GC: EI, CI, PI, FI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC: ESI, APCI</td>
</tr>
</tbody>
</table>

**Topic 4**

**Analysis of samples insoluble in solvents**

Samples that are insoluble or dispersed in solvents are difficult to analyze in GC or LC. Direct sample loading is effective for such samples.

Direct sample loading, designed to ionize an entire sample introduced into a mass spectrometer, is effective for analysis of unmixed compounds. For analysis of mixed samples, soft ionization makes it possible to identify components from molecular ions and cation clusters. Direct loading methods supported by JEOL mass spectrometers are listed below.

| DEI, DCI, FD, MALDI, DART |
Electron Ionization (EI)

This is the ionization method most widely used for GC-MS. It is designed to produce ions by irradiating thermal electrons emitted from a filament to gaseous sample molecules. It is easily combined with GC because sample molecules need to be in the form of gas to be ionized. EI, being the hardest ionization technique, produces many fragment ions. Because the relative intensity of each ion observed (spectral pattern) has high reproducibility, components are easily identified by comparing the acquired spectrum against the EI mass spectrum database. The database contains more than 200,000 compounds, supporting various GC-MS applications.

Electron ionization (EI)

EI application: NIST library search

Result of NIST library search for octafluoronaphthalene


Result of NIST library search for octafluoronaphthalene
Chemical Ionization (CI)

This is a typical ionization method used for GC-MS. A reagent gas is introduced into a highly airtight ionization chamber (approx. $10^{-2}$ Pa), and the reagent gas (B) is ionized by thermal electrons. Sample molecules (M) are then introduced to produce protonated molecules through the ion molecular reaction with the reagent gas ions. Compared to EI, CI is a soft ionization method, one of the techniques effective in determining the molecular weight of an unknown sample. For the reagent gas, methane, isobutane, and ammonia are primarily used. When methane and isobutane reagent gases are used, $[M+H]^+$ ions are observed; when an ammonia gas is used, $[M+NH_4]^+$ ions are observed.

Chemical Ionization (CI) application: Qualitative analysis of an unknown compound

<table>
<thead>
<tr>
<th>Mode</th>
<th>Obs. m/z</th>
<th>Error (mDa)</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI+</td>
<td>69.0715</td>
<td>1.1</td>
<td>C_8H_9</td>
</tr>
<tr>
<td></td>
<td>111.1187</td>
<td>1.3</td>
<td>C_9H_11</td>
</tr>
<tr>
<td></td>
<td>195.0689</td>
<td>1.4</td>
<td>C_9H_11NO</td>
</tr>
<tr>
<td></td>
<td>233.1743</td>
<td>1.4</td>
<td>C_9H_11NO_2</td>
</tr>
<tr>
<td>CI+</td>
<td>334.1802</td>
<td>-0.5</td>
<td>C_9H_11NO_2</td>
</tr>
</tbody>
</table>

Unknown compound in liquid crystal mixture
Mass spectra of EI and CI

Exact mass results and Estimated chemical formula
Photo Ionization (PI)

Photo ionization is designed to irradiate vacuum ultraviolet (VUV) light inside an ionization chamber to provide light energy at 8 to 10 eV to sample molecules. It is a soft ionization method featuring low ionization energy, suppressing fragmentation, because the ionization energy for typical organic compounds is 8 to 11 eV. Compounds that are absorbed in the ultraviolet region, such as aromatic compounds, tend to have higher sensitivity compared to other compounds. Photo ionization is one of the soft ionization methods for GC-MS that require no reagent gas. It is easy to use and is effective in estimating the molecular formula of an unknown compound.

PI application: Analysis of aromatic compounds

### Polycyclic aromatic hydrocarbons

- **M**
  - C₇H₈ (1-ring) m/z 92.093
  - C₁₁H₁₀ (2-ring) m/z 142.078
  - C₁₅H₁₂ (3-ring) m/z 192.064

- **M**
  - Toluen m/z 92.093
  - Methylchrysalene m/z 143.081
  - Methylanthracene m/z 193.098
FD and FI are soft ionization methods developed to support analysis of thermally unstable compounds, refractory compounds, and high molecular weight compounds. Both apply a potential difference of 8 to 10 kV to an emitter, and ionize the sample as electrons in sample molecules move to the emitter by the tunneling effect. FI refers to the process in which vaporized sample molecules are ionized as they pass near the emitter while FD refers to the process in which a sample is applied onto the emitter and is ionized while being heated. FI is used for GC, and FD for direct loading. The energy given for ionization by FD or FI is 1 eV or less, and the internal energy of ionized molecular ions is fairly low compared to EI and CI. Therefore, FD and FI are soft ionization with little or no fragmentation. FD and FI, both being soft ionization methods, are used for molecular ion detection. FD is particularly effective for thermally unstable substances and refractory substances.

**Field Ionization / Field Desorption (FI / FD)**

FD and FI are soft ionization methods developed to support analysis of thermally unstable compounds, refractory compounds, and high molecular weight compounds. Both apply a potential difference of 8 to 10 kV to an emitter, and ionize the sample as electrons in sample molecules move to the emitter by the tunneling effect. FI refers to the process in which vaporized sample molecules are ionized as they pass near the emitter while FD refers to the process in which a sample is applied onto the emitter and is ionized while being heated. FI is used for GC, and FD for direct loading. The energy given for ionization by FD or FI is 1 eV or less, and the internal energy of ionized molecular ions is fairly low compared to EI and CI. Therefore, FD and FI are soft ionization with little or no fragmentation. FD and FI, both being soft ionization methods, are used for molecular ion detection. FD is particularly effective for thermally unstable substances and refractory substances.

**Field ionization / Field desorption (FI/FD)**

**FI/FD application:** Molecular ions detected in a low-polarity compound and a refractory compound

- **n-Tetradecane (C_{14}H_{29}O)**
  - Mass spectrum of n-tetradecane
  - Top: EI; bottom: FI

- **Pigment red 144 (C_{21}H_{22}C_{12}(N_{2}O_{5})**
  - Mass spectrum of pigment red 144
  - Top: DEI; bottom: FD
Electrospray Ionization (ESI)

This is the most widely used ionization method using an electrospray. A sample solution is injected into a capillary, and is ionized by applying high voltage between the capillary and its corresponding electrode (orifice electrode). Using the lowest ionization energy among all ionization methods, ESI is the only ionization method that acquires molecular weight information from biological polymer samples as well as coordinate bond compounds including complexes. When used for proteins, peptides, and nucleic acids, ESI acquires multiply charged ions.

Cold-Spray Ionization (CSI)

CSI is a low temperature ionization method using a cold nitrogen gas to spray the ESI ion source. It is effective in ionizing biological molecules, reaction intermediates, host-guest compounds, and various aggregates in solutions in addition to metal complexes that are difficult to analyze by ESI.

ColdSpray ionization was developed by Prof. Kentaro Yamaguchi, et al., of Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University (previously Chiba University) and the result of a project funded by the Japan Science and Technology Agency (JST). Reference: Cold-Spray ionization mass spectrometry: principle and applications K. Yamaguchi, J. Mass Spectrom, 38, 473-490 (2003)

ORTEP diagram of the complex [Cu$_2^{2+}$ L3$_6$ (PhOPO$_3^{2-}$)$_4$].

(Data courtesy of Prof. Masahito Kodera, Doshisha University)
A typical ambient ionization method, DART™ ionizes samples in atmosphere without preliminary treatment. The ionization process of DART™ is based on the interaction of excited atoms/molecules with an atmospheric gas and sample. A helium gas introduced to DART™ produces a plasma through the discharge of a needle electrode. The plasma contains ions, electrons, excited (quasi-stable) atoms and molecules. Most of the charged particles in the plasma are removed by a ground electrode, emitting only the excited neutral gas molecules to atmosphere. The gas is heated as needed to promote vaporization of the sample and thermal desorption from the substance surface.

**Positive ions**

Excited helium atoms react to water in atmosphere, producing protonated water clusters. These react to an analyte (M), producing protonated molecules.

\[
\text{He}^{(2S)} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}^- + \text{He}^{(1S)} + e^-
\]

\[
\text{H}_2\text{O}^- + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}^+ + \text{OH}^- + (n+1)\text{H}_2\text{O}
\]

**Negative ions**

Excited helium atoms react to an exit grid electrode and neutral atmosphere (N), producing electrons through Penning ionization. These electrons collide with gas (G) in atmosphere, rapidly losing speed, and react with oxygen in atmosphere, producing negative oxygen ions.

\[
\text{He}^{(2S)} + N_2 \rightarrow N^- + \text{He}^{(1S)} + e^-
\]

\[
e^- + \text{G} \rightarrow \text{G}^- + e^-
\]

\[
e^- + \text{O}_2 \rightarrow \text{O}_2^-
\]

These negative ions further react to sample molecules (M), producing negative ions of the analyte.

\[
\text{O}_2^- + M \rightarrow [M - \text{H}]^-
\]

\[
\text{O}_2^- + M \rightarrow [M + \text{OH}]^-
\]

\[
\text{O}_2^- + M \rightarrow [M + \text{O}_2^-]
\]
MALDI is a typical soft ionization method besides electrospray ionization (ESI). A matrix, which is a solution to accelerate ionization, is mixed with a sample solution, and the mixture is dribbled onto a stainless steel plate. As it dries, the mixture forms a cocrystal, to which ultraviolet laser is irradiated, ionizing sample molecules. By selecting an appropriate matrix for the sample, MALDI can ionize low to high molecular weight samples. Because singly charged ions are primarily produced, the horizontal axis equals the mass of molecule, making it easy to interpret mass spectral data.
Some of the low molecular compounds can be ionized without a matrix. This type of ionization process is specifically called laser desorption ionization. Typical compounds subjected to this ionization method include polycyclic aromatics, pigments/dyes, and organic electronic parts (organic EL parts).

- **Sample extracted from deposits at Tokyo Bay with dichloromethane**

Laser desorption ionization also supports mass imaging that maps organic substances on the sample surface. A sample section is placed onto a conductive plate (ITO slide glass), and coated with a matrix. The sample is subjected to laser irradiation at an interval of 10 to 100 µm to acquire mass spectral data.

- **Lipid distribution on a mouse brain section**

![Graphical representation of lipid distribution](image-url)