

Liquid Chromatograph Mass Spectrometer

# LCMS-IT-TOF



# LCMS-IT-TOF

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## High-Speed Liquid Chromatograph Mass Spectrometer LCMS-IT-TOF™

A significantly advanced MS detector enabling  
LC/MS<sup>n</sup> high-precision mass analysis



High speed and high precision in MS<sup>n</sup> measurement can  
provide researchers with a host of new possibilities.

## High Resolution & High Precision in MS<sup>n</sup> measurement

The stable, high-resolution spectra achieved throughout a wide mass range and high-precision MS<sup>n</sup> data are derived from the DSR: Dual-Stage Reflectron <sup>(\*1)</sup> and the use of BIE: Ballistic Ion Extraction <sup>(\*2)</sup>. These instrument advances can strongly assist in the use of MS for predicting accurate structural details.

## High Throughput

Increased amounts of information are obtained for each measurement, enabling much higher reliability in structural analysis. High-throughput analysis can be realized with the world's best performance hybrid MS used for structural analysis in terms of high-speed mass spectrum measurement and high-speed ion polarity switching.

## High Sensitivity

High-sensitivity detection of low concentration samples is derived through the use of Compressed Ion Injection (CII) within the ion optics <sup>(\*3)</sup>, allowing for the ions to be placed efficiently into the ion trap.

\*1 Patent US6384410, US6803564 and others

\*2 Patent US6380666 and others

\*3 Patent US6700116 and others

## Contents

P 04 - Original and Advanced Technology

P 06 - High-Speed and High-Precision MS<sup>n</sup> Measurement

P 08 - Basic Performance Supporting High-Quality Data

P 10 - Intelligent Auto MS<sup>n</sup> Function

P 12 - Software to Accelerate Data Analysis

# Original and Advanced Technology

## What is the IT-TOF?

There are several types of LC/MS instruments in the market currently. The single quadrupole and triple quadrupole instruments are primarily used for quantitative analysis, while the ion trap, LC-TOF and Qq-TOF instruments are primarily used for qualitative analysis. Triple quadrupoles are excellent instruments for quantitative analysis, although they falter in comparison to other types of mass spectrometers in terms of mass accuracy and resolution. Ion traps excel in structural analysis due to their ability to perform MS<sup>n</sup>; however, the mass accuracy and resolution, like with a triple quadrupole MS, are limited. On the other hand, the Qq-TOF fea-

tures excellent resolution and mass accuracy compared to the above-mentioned types of instruments. They do exhibit a downside in that it is not possible for Qq-TOF instruments to perform MS<sup>3</sup> or greater as their construction does not support MS<sup>n</sup>.

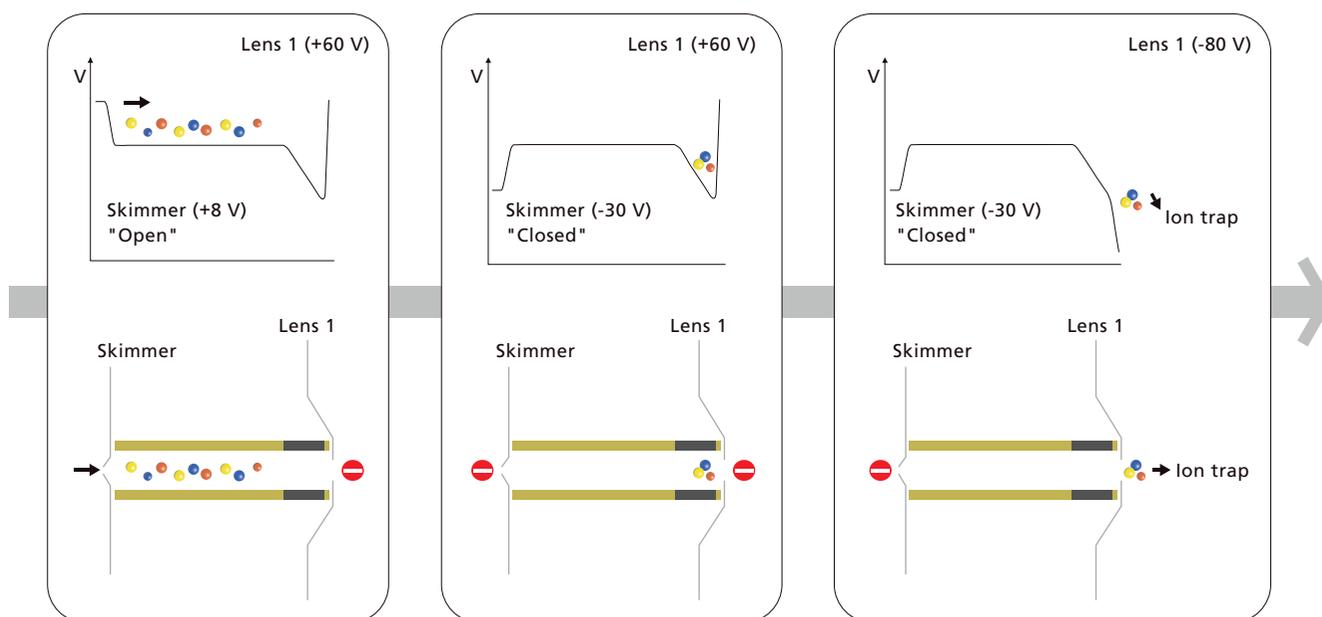
Shimadzu developed a new type of hybrid mass spectrometer, the LCMS-IT-TOF, which possesses both the MS<sup>n</sup> ability of an ion trap and the excellent resolution and mass accuracy of a TOF.

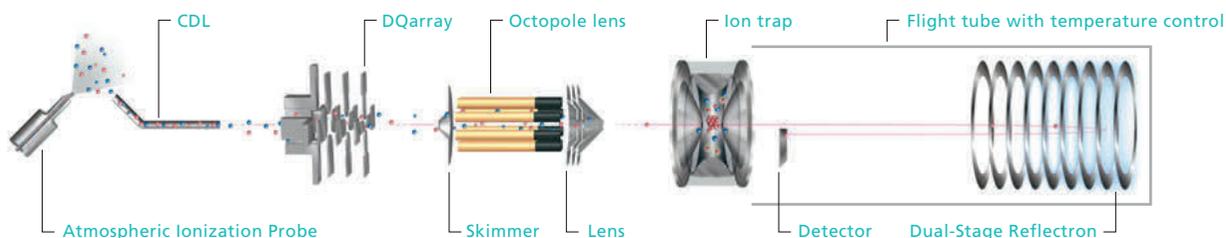
The LCMS-IT-TOF opens new doors to the prediction of elemental composition and structural analysis.

## Compressed Ion Introduction (CII™)

The ion optical system used in the LCMS-IT-TOF leads to a novel ion introduction method referred to as Compressed Ion Introduction or CII, where the combination of the skimmer, octopole and first lens converts the continuous stream of ions into pulses for introduction into the ion trap. This method makes it possible to control the accumulation of ions before they are introduced into the ion trap, allowing the RF to be applied to the ring electrode at the instant that

all of the CII-accumulated ions enter the ion trap. This new method of controlling the ion trap, which is quite different from a traditional ion trap, is adopted for the LCMS-IT-TOF. The development of this CII effectively couples the LC system to the MS and enables a dramatic improvement over the previously deficient ion capture rate of the ion trap, thereby increasing sensitivity.





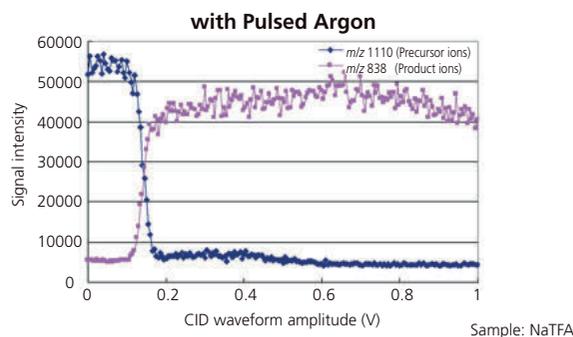
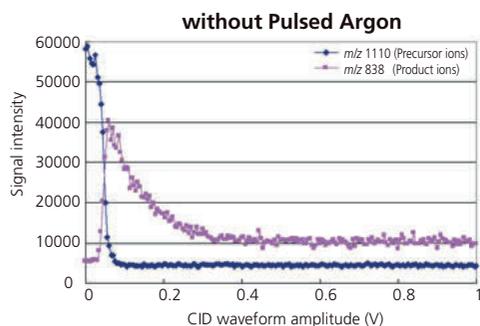
In the LCMS-IT-TOF, rather than simply combining the existing technologies of the QIT (Quadrupole Ion Trap) mass spectrometer and an oaTOF (Orthogonal Acceleration Time of Flight) mass spectrometer, the QIT and TOF are arranged linearly in a unique construction. This construction offsets the disadvantages of slow measurement

speed, ion capture rate and trap saturation associated with the ion trap, while allowing the advantages of the ion trap and TOF to be fully demonstrated. Abundant qualitative information can be obtained within a limited HPLC peak elution time.

## High-Efficiency Collision-Induced Dissociation using Argon

### Pulsed Ar CID

In the LCMS-IT-TOF, Ar gas is introduced into the trap via a pulse valve to perform cleaning just prior to conducting CID (Collision-Induced Dissociation). This introduction of Ar has resulted extremely efficient CID.



Sample: NaTFA

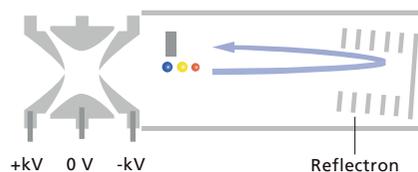
Without pulsed Ar introduction, product ion intensity noticeably decreases as the CID energy is increased. On the other hand, with pulsed Ar introduction, the product ion intensity remains unchanged even as the CID energy increases. In the case of traditional ion traps, it is necessary to adjust the CID energy

depending on the  $m/z$  value selected; however, with the LCMS-IT-TOF, equipped with the unique technology of pulsed Ar CID, MS<sup>n</sup> measurement of compounds having various  $m/z$ 's can be performed under a single set of CID conditions.

## Addressing Sharp Chromatographic Peaks...

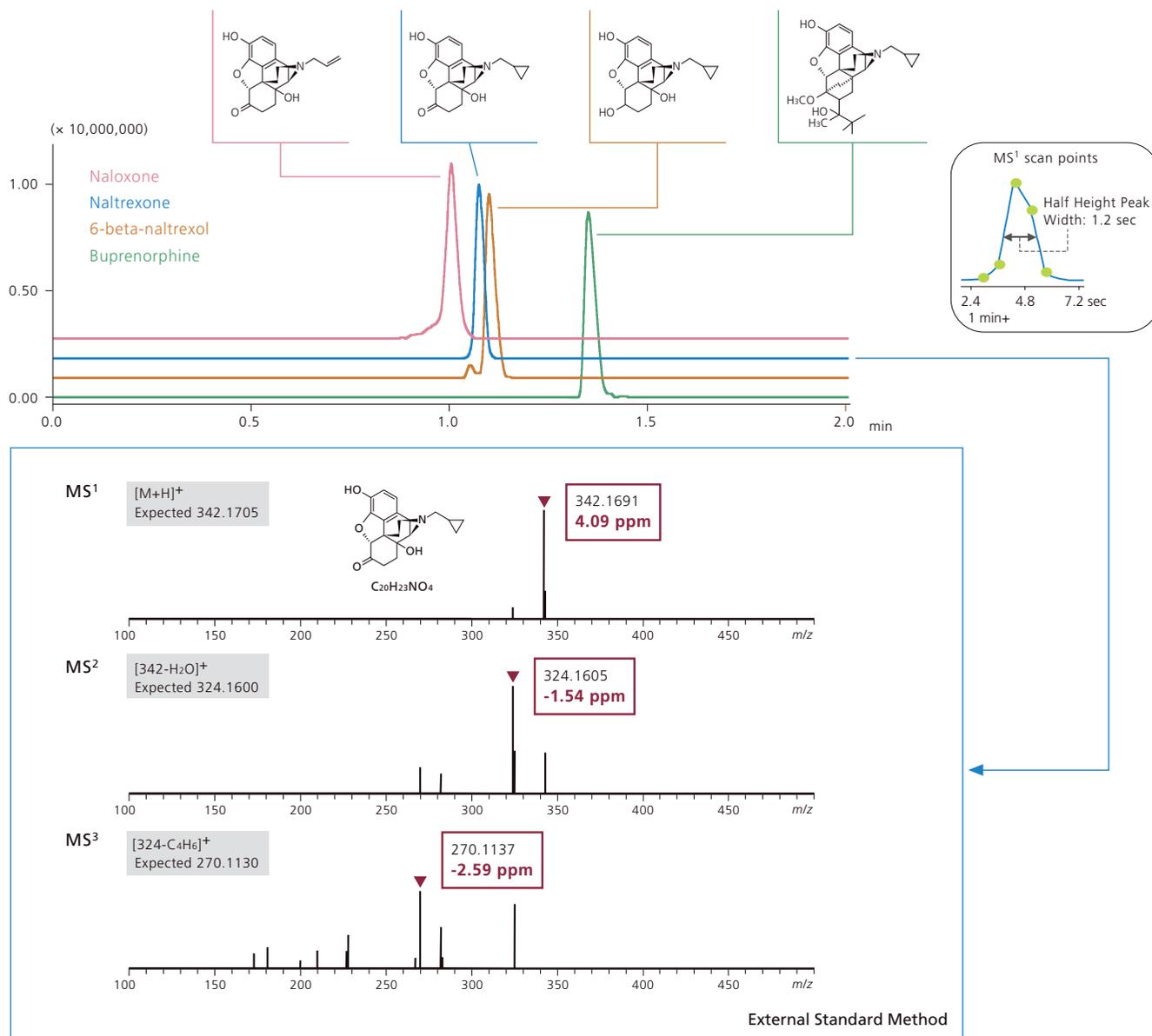
### BIE [Ballistic Ion Extraction]

In the LCMS-IT-TOF, faster spectrum measurement has been achieved by accelerating the ions from the ion trap to the TOF using a new technology termed Ballistic Ion Extraction (BIE), which enables high-throughput analysis. BIE is an ion accelerating method to inject ions into the TOF instantly by applying a high voltage with opposite polarity to the end-caps and lowering the ring electrode RF voltage to 0V. BIE aids in lowering the spatial distribution of the ions as they enter the TOF region.



# High-Speed and High-Accuracy MS<sup>n</sup> Measurement

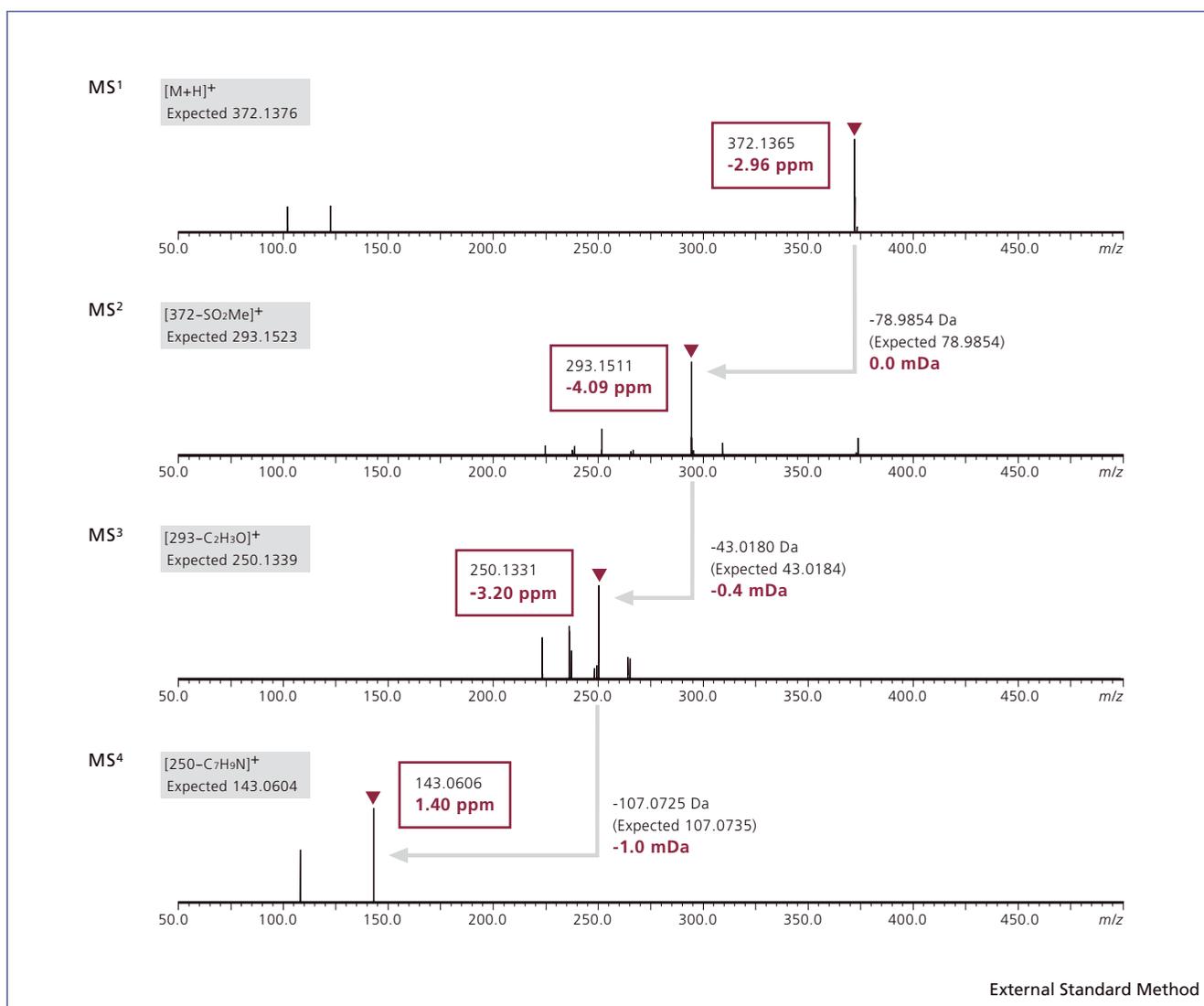
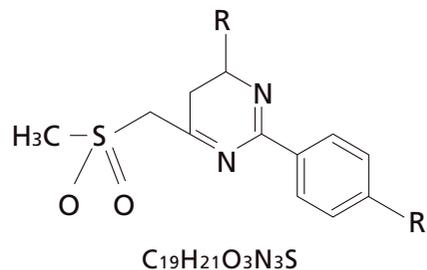
The LCMS-IT-TOF is intended to strongly assist in the identification of target compounds by using high-speed/high-accuracy MS<sup>n</sup> data in R&D fields such as impurity analysis, metabolic profiling and biomarker research.



The data above shows the analysis of four compounds within 2.0 minutes. The Auto MS<sup>n</sup> function makes it possible to obtain highly accurate MS<sup>n</sup> data via an external standard, taking advantage of high-speed mass spectrum measurement performance.

Column	: Shim-pack XR-ODS (2.0 × 30 mm, 2.2 μm)
Mobile phase A	: 0.1 % formic acid
Mobile phase B	: 0.1 % formic acid in methanol
Time program	: 4 % B (0 min) – 100 % B (0.5 min) – 4 % B (0.51– 2.00 min)
Flow speed	: 0.5 mL/min
Injection volume	: 5 μL (1 ng/μL)
Column oven temperature	: 50°C

Ion fragmentation is very useful in structural analysis. The LCMS-IT-TOF has a unique design in that it connects an ion trap with a TOF and provides high-precision mass information for either MS or MS<sup>n</sup>. This makes it possible to perform highly reliable structural analysis.



The above examples show that the low-mass product ions can be analyzed precisely and the new compounds can be identified by MS<sup>n</sup> measurement.

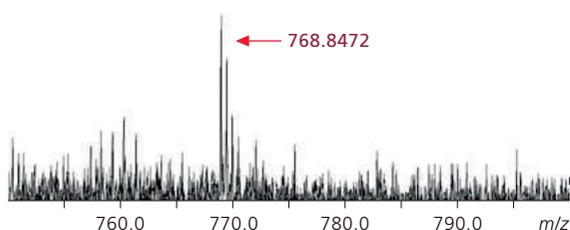
Sample provided by: AstraZeneca (UK) Dr. Richard Gallagher

# Basic Performance Supporting High-Quality Data

## Compatibility of High Sensitivity and Mass Accuracy

100 attomol of Fibrinopeptide A

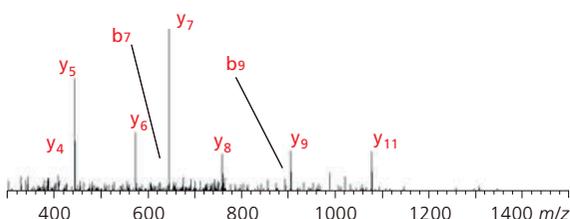
MS<sup>1</sup>



M.W. 1535.6852  
ADSGEGDFLA EGGV R

Ion	Measured	Expected	Error (ppm)
[M+2H] <sup>2+</sup>	768.8472	768.8506	-4.4
y4	388.2332	388.2308	6.2
y5	445.2506	445.2523	-3.8
y6	574.3000	574.2949	8.9
y7	645.3255	645.3320	-10.1
y8	758.4197	758.4161	4.7
y9	905.4801	905.4845	-4.9
y11	1077.5442	1077.5329	10.5
b7	632.2098	632.2164	-10.4
b9	892.3636	892.3688	-5.8

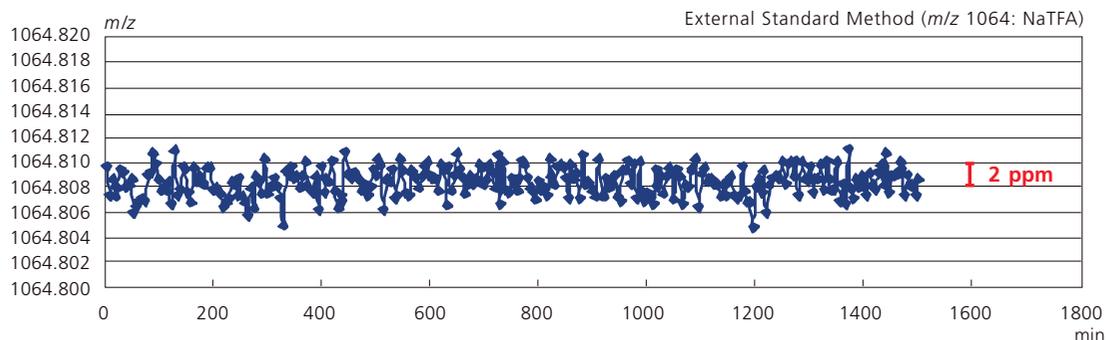
MS<sup>2</sup>



## Extended Stability in Mass Accuracy

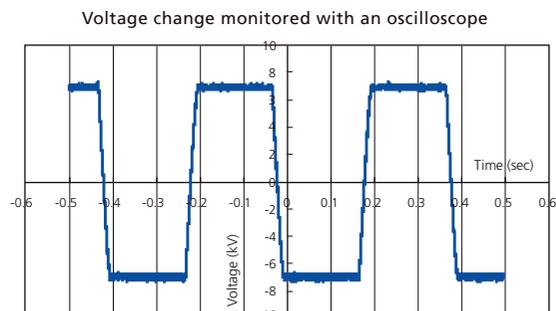
The high-accuracy temperature control mechanism of the flight tube, the localized ion due to BIE from the QIT, and the effective 10-bit high-speed transient recorder enable extremely stable mass accuracy using the external standard method. Due to labor-intensive work required when using an internal standard for LC/MS anal-

ysis, it is extremely important to achieve stable high mass accuracy over a wide mass range using an external standard. In the LCMS-IT-TOF, TOF mass calibration can be automatically performed to maintain the stability for an extended period of time.



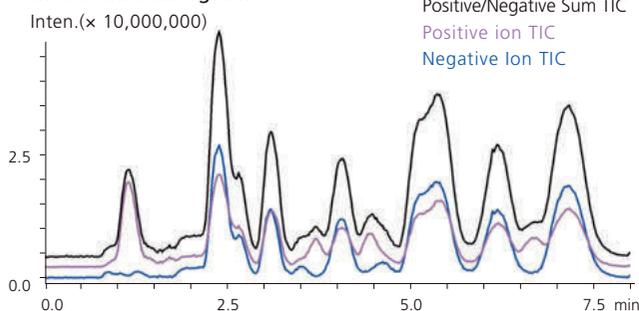
## High-Speed Ion Polarity Switching Mode [up to 2.5 Hz]

High-Speed Ion Polarity Switching can be especially useful when it cannot be judged whether samples will be detected as positive or negative ions. The LCMS-IT-TOF utilizes a newly developed, highly accurate and stable power supply as well as a newly developed high-voltage switch that allows for polarity switching in only 0.1 sec or less (necessary for the sharp HPLC peaks available with the advances in high-speed chromatography). The maximum rate for polarity switching is 2.5 Hz, which allows for a pair of positive and negative ion MS spectra to be obtained 2.5 times per second.



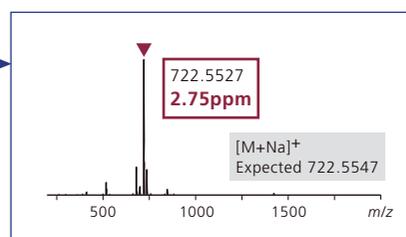
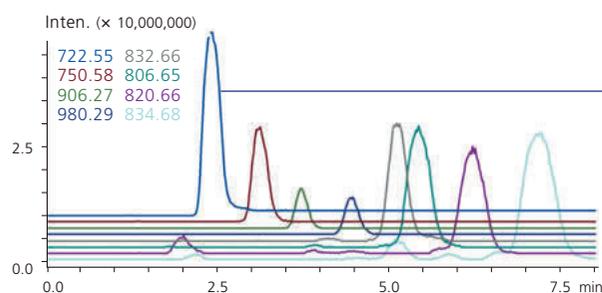
## High-Speed Ion Polarity Switching Mode Measurement Example

### Total Ion Chromatogram

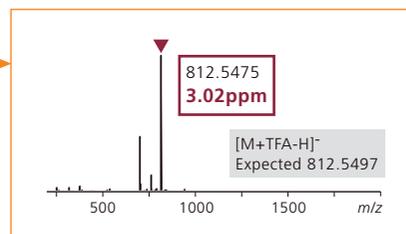
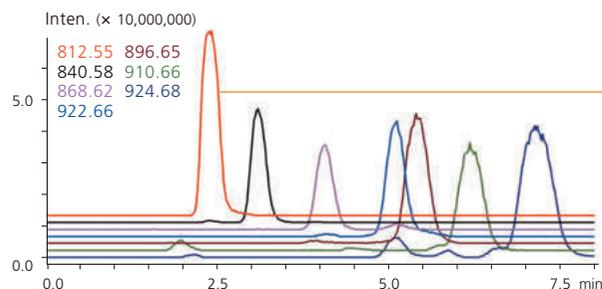


Sample	: glycosylceramide (C16,18,20,22,23,24:0-d18:1), (C24:1-d18:1)
Column	: Imtakt Cadenza CD-C18
Mobile phase	: 5 mM Acetic acid in methanol
Flow rate	: 0.2 mL/min

### Positive Ion Mass Chromatogram



### Negative Ion Mass Chromatogram



Provided by RIKEN

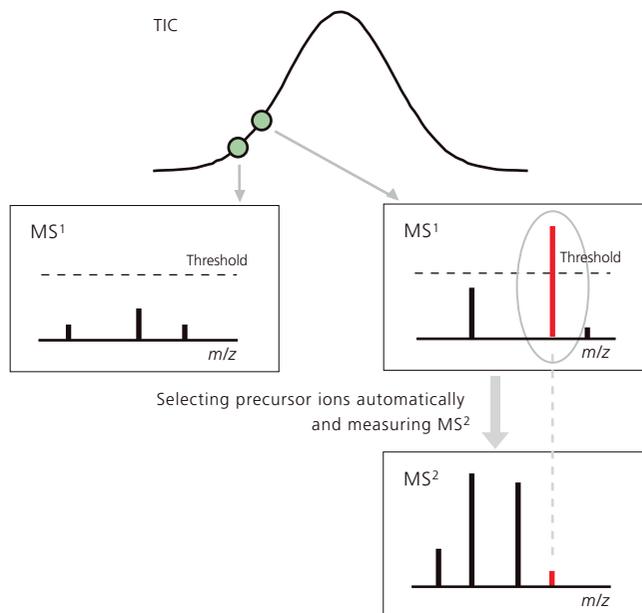
# Intelligent Auto MS<sup>n</sup> Function

## What is the Auto MS<sup>n</sup> Function?

Samples cannot be recaptured once injected for LC/MS analysis; therefore, it is vital that instruments can automatically select the appropriate precursor ions. With the LCMS-IT-TOF, a variety of precursor ion selection criteria, such as the selection of ions in order of intensity or  $m/z$  is available, in addition to intelligent automatic precursor selection, such as a monoisotopic peak selector and charge-state filtering.

### Main Functions for Precursor Ion Selection

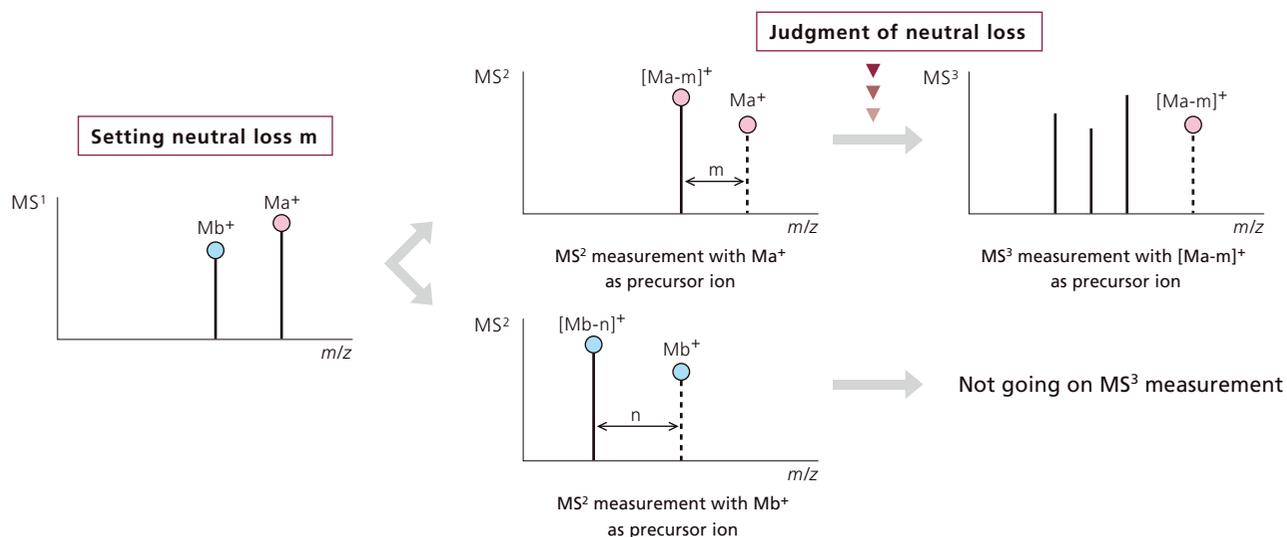
- ▮ Selection in the order of intensity or  $m/z$
- ▮ Charge state filter
- ▮ Monoisotopic filter
- ▮ Exclusion ion (Automatic exclusion)
- ▮ Pair peak selection
- ▮ Preference Ion Selection
- ▮ Neutral Loss Survey



## Principles of Neutral Loss Survey

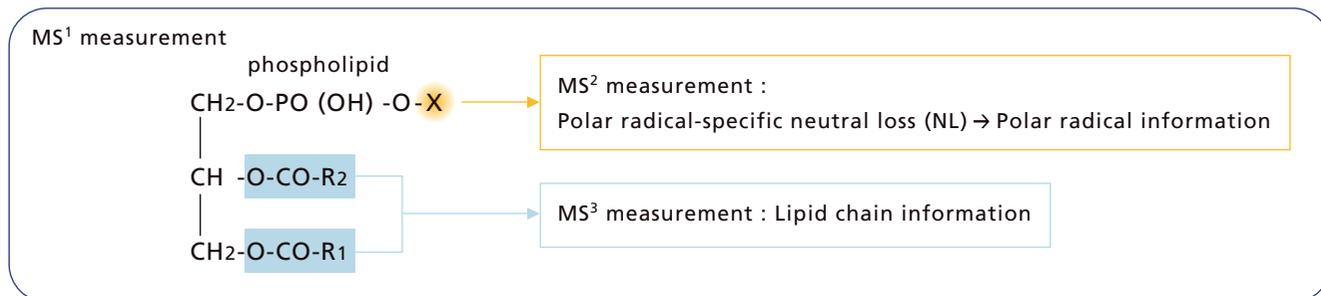
MS<sup>3</sup> measurements are automatically performed if the specified neutral loss is observed in the MS<sup>2</sup> spectrum. With the Neutral Loss Survey, only the target ions are measured in MS<sup>3</sup>, enabling one to obtain desired information effectively without loss of time. As de-

tailed information about target ions is obtained using the neutral loss survey function, it can become a powerful tool for supporting the identification of compounds (e.g., phase II metabolites for drug discovery research).



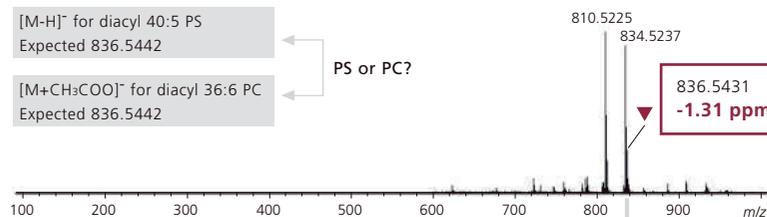
## Examples Highlighting the Neutral Loss Survey Function

The combination of the neutral loss survey and MS<sup>3</sup> measurement provides accurate mass information that can lead to highly reliable structural analyses of phospholipids.

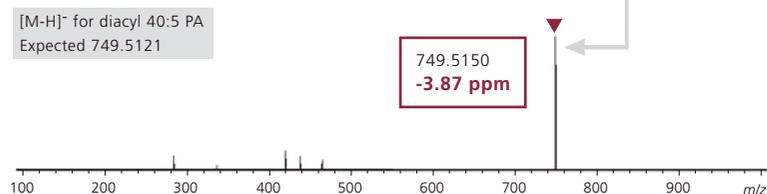


### Setting PS (Polar radical X : serine) - specific NL (87 Da) and performing the neutral loss survey

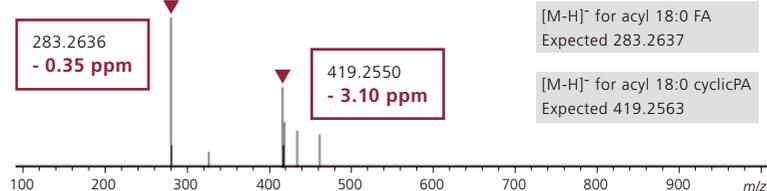
MS<sup>1</sup>



MS<sup>2</sup> of m/z 836.5



MS<sup>3</sup> of m/z 749.5

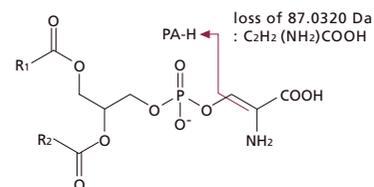


PC : Phosphatidilcholine

PS : Phosphatidilserine

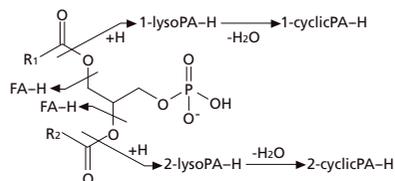
PA : Phosphatidic acid

FA : Fatty acid



Detection of PS-specific NL: Polar radical information

Performing MS<sup>3</sup> measurement of precursor ions  
at m/z 749 by neutral loss survey



MS<sup>3</sup> measurement: Lipid chain information

Sample : lipid mixture from rat liver  
Column : Si column (1.0 × 10 mm)  
Mobile phase : gradient of acetonitrile (A)  
and methanol (B)  
(including 0.3 % formic  
acid and 0.1 % ammonia  
water in (A) and (B) each)

Using neutral loss survey, diacyl 18:0 – 22:5 PCs in the lipid mixture were easily identified. Mass accuracy of less than 5 ppm was obtained in MS<sup>1</sup> through MS<sup>3</sup> using an external standard.

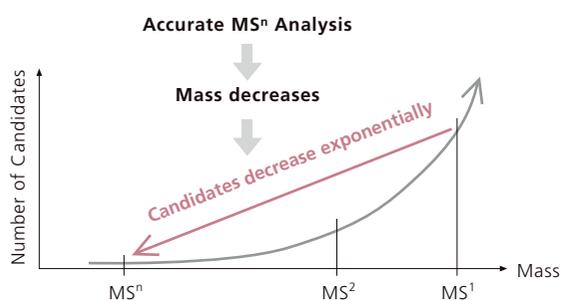
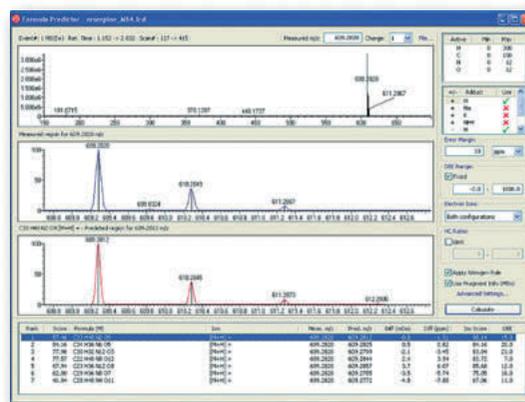
# Optional Software for Accelerating Data Analysis

## Formula Predictor

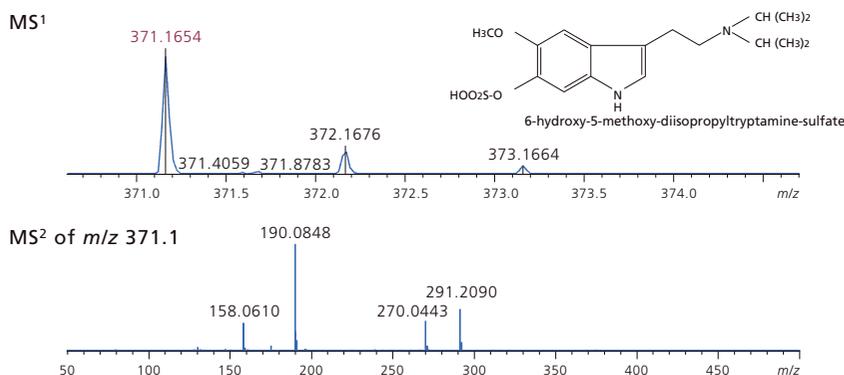
### Effectiveness of Accurate MS<sup>n</sup>

In composition prediction, target constituents having a small mass and high mass accuracy associated with their measured values, provide fewer numbers of candidates and greater prediction reliability.

When using MS<sup>2</sup> data with Formula Predictor, formula prediction starts with the product ion having the smallest mass, and uses that result in the effective prediction of the parent ion by reducing the number of candidates.



### C<sub>17</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S+H<sup>+</sup> (m/z 371.1641) Composition Prediction Example



Take, for example, the case of predicting the elemental composition of the ion at m/z 371.1645.

The correct composition is C<sub>17</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S+H<sup>+</sup> (m/z 371.1641).

The composition prediction procedure is as follows:

#### (1) Composition Calculated from Mass

When simply calculating the composition only from the mass value, the proper composition is ranked fifth.

#### (2) Using Isotopic Pattern

When additionally comparing and ranking the isotopic patterns, the correct composition is ranked first.

#### (3) MS<sup>n</sup> Spectral Filtering

Finally, taking into account the MS<sup>n</sup> data enables 12 candidates to be reduced to 2, as shown in the table.

(1) Composition Calculated from Mass		(2) Using Isotopic Pattern		(3) MS <sup>n</sup> Spectral Filtering	
#	Formula	#	Formula	#	Formula
1	C <sub>18</sub> H <sub>22</sub> N <sub>6</sub> O <sub>5</sub> S	1	<b>C<sub>17</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S</b>	1	<b>C<sub>17</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S</b>
2	C <sub>10</sub> H <sub>18</sub> N <sub>12</sub> O <sub>4</sub>	2	C <sub>10</sub> H <sub>18</sub> N <sub>12</sub> O <sub>4</sub>	2	C <sub>6</sub> H <sub>26</sub> N <sub>8</sub> O <sub>8</sub> S
3	C <sub>25</sub> H <sub>22</sub> O <sub>3</sub>	3	C <sub>18</sub> H <sub>22</sub> N <sub>6</sub> O <sub>5</sub> S		
4	C <sub>13</sub> H <sub>26</sub> N <sub>2</sub> O <sub>10</sub>	4	C <sub>9</sub> H <sub>22</sub> N <sub>8</sub> O <sub>8</sub>		
5	<b>C<sub>17</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S</b>	5	C <sub>13</sub> H <sub>26</sub> N <sub>2</sub> O <sub>10</sub>		
6	C <sub>9</sub> H <sub>22</sub> N <sub>8</sub> O <sub>8</sub>	6	C <sub>25</sub> H <sub>22</sub> O <sub>3</sub>		
7	C <sub>6</sub> H <sub>26</sub> N <sub>8</sub> O <sub>8</sub> S	7	C <sub>6</sub> H <sub>26</sub> N <sub>8</sub> O <sub>8</sub> S		
8	C <sub>14</sub> H <sub>22</sub> N <sub>6</sub> O <sub>6</sub>	8	C <sub>8</sub> H <sub>26</sub> N <sub>4</sub> O <sub>12</sub>		
9	C <sub>22</sub> H <sub>26</sub> O <sub>3</sub> S	9	C <sub>14</sub> H <sub>22</sub> N <sub>6</sub> O <sub>6</sub>		
10	C <sub>8</sub> H <sub>26</sub> N <sub>4</sub> O <sub>12</sub>	10	C <sub>22</sub> H <sub>26</sub> O <sub>3</sub> S		
11	C <sub>7</sub> H <sub>22</sub> N <sub>12</sub> O <sub>4</sub> S	11	C <sub>21</sub> H <sub>18</sub> N <sub>6</sub> O		
12	C <sub>21</sub> H <sub>18</sub> N <sub>6</sub> O	12	C <sub>7</sub> H <sub>22</sub> N <sub>12</sub> O <sub>4</sub> S		

In this way, using the isotopic patterns enables the correct formula to achieve a higher ranking. Furthermore, using MS<sup>n</sup> data enables a reduction in the number of candidates.

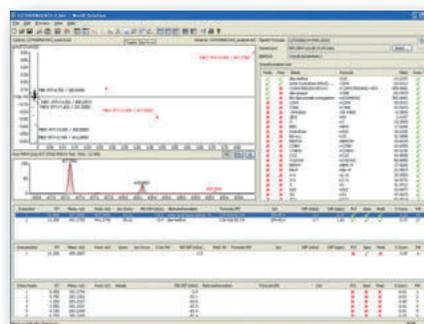
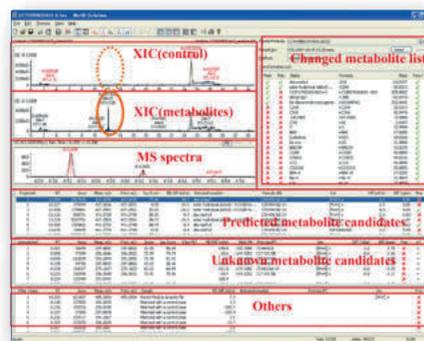
## MetID Solution

### Comprehensive Metabolite Detection and Structure Prediction Using Accurate Mass Information from HPLC and Online MS<sup>n</sup>

This software compares data from an unmetabolized control sample and a metabolized target sample to detect metabolites, and the built-in Composition Prediction Software predicts the composition. Peaks existing in the target sample data but not in the control sample data are possible metabolites. Metabolite candidates are accurately selected through comparison of their isotopic patterns and these are used to support identification. Comprehensive metabolite candidate information can also be acquired for metabolites with an unknown pathway by exploiting the high-speed data measuring capacity of the LCMS-IT-TOF to conduct MS<sup>n</sup> measurements of accurate mass information with online HPLC, and then applying unique multivariate analysis.

Moreover, an isotope filter chromatogram (IFC) function is available to extract required data from the huge amount of information. IFC is useful for the detection of compounds labeled with stable isotopes or radio isotopes and assists with the detection of reactive metabolites. More generally, it is also effective for highly reliable detection of compounds containing elements with characteristic isotopic patterns, such as chlorine or bromine.

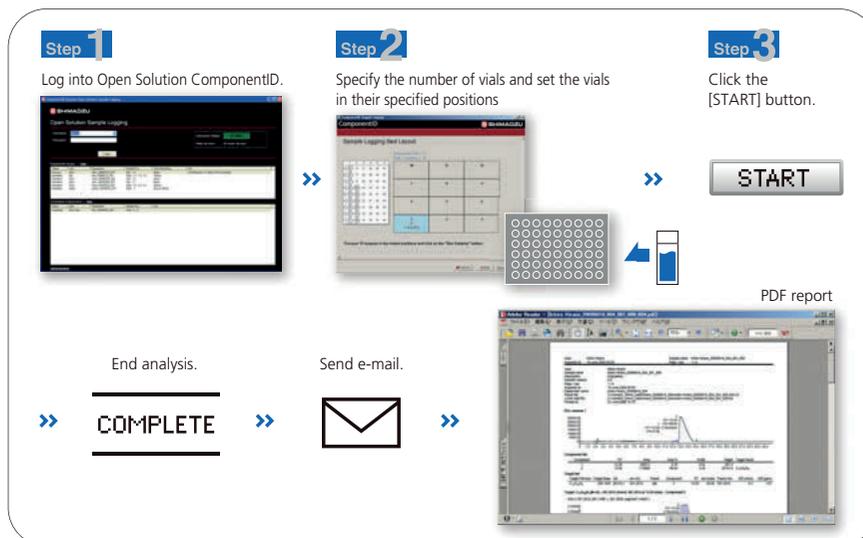
The MetID Solution approach of applying multivariate analysis to exhaustively detect compounds with a structure resembling the major components is also extremely effective for the efficient analysis of impurities and natural products in synthetic compounds.



## Open Solution ComponentID

### Fully Automated, from Precision Mass Spectrometry Measurements to Formula Prediction and Report Creation

Open Solution ComponentID is a software tool developed in response to customer requests for open access software that allows performing precision mass spectrometry measurements using LCMS-IT-TOF, without relying on specialized operators. It enables running analyses in only 3 steps. After measurements are finished, it automatically sends a report of formula prediction results to the email address of the user currently logged in.



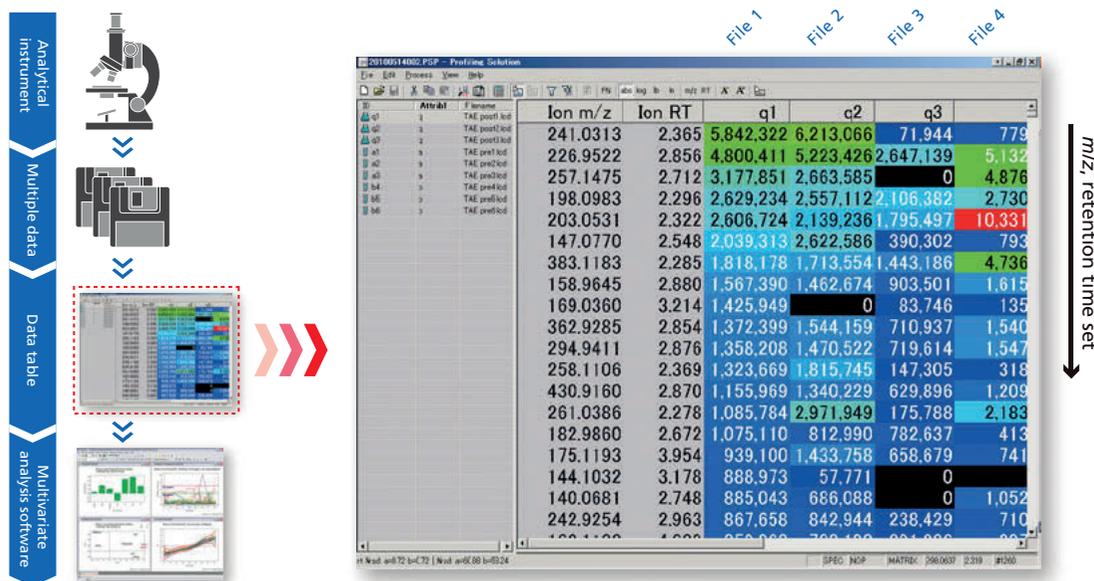
# Software to Accelerate Data Analysis

## Profiling Solution Ver. 1.1

### Creates Data Array by Extracting Peaks from Multiple Data Files

This software automatically detects peaks in multiple data files analyzed using the LCMS-IT-TOF, performs any additional operations, such as listing peak retention times, as necessary, and then tabulates results. This step of creating a table from measurement data is a critical step that can determine the success or failure of metabolomics or differential analysis for food products, cosmetics, or other

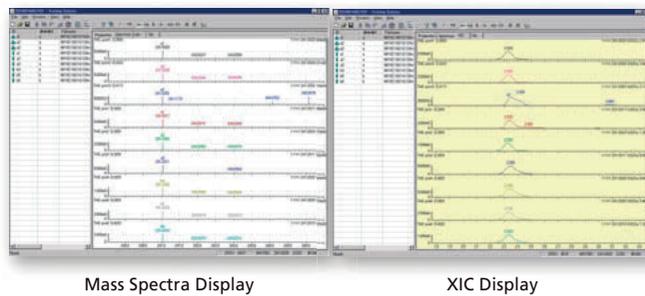
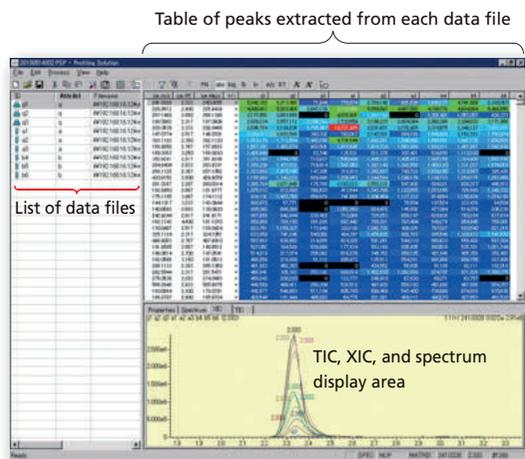
fields. Original data can be easily referenced from the table, which helps to ensure highly reliable data analysis. Tabulated data can be analyzed using commercial statistical analysis software, which enables utilizing a wide variety of analytical techniques. This is especially useful for metabolomics or impurities profiling applications using LCMS-IT-TOF, which enables high-speed analysis.



### Automatic Extraction and Display of Peaks from a Large Number of Data Files

Simply drag and drop multiple measurement data files and click the run button to output the tabulated content of data files. This tabulated data can be used as data for multivariate analysis.

- Chromatograms and mass spectra can be displayed for all data.
- Operations (division, integration, logic operations) and filtering functions for the signal intensities are provided to allow comparison and overview of the data.



## Protein Layer

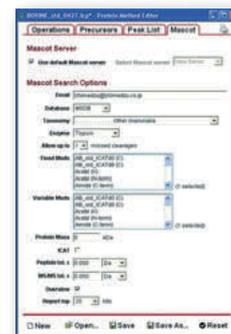
By combining LCMSsolution with the newest addition to the solutions suite, Protein Layer, the LCMS-IT-TOF can be set up for automated protein identification experiments.

Protein Layer features the same user-friendly interface as LCMSsolution.

By setting the peak-list generation and database search parameters in the protein method, the process can be automated from analysis to protein identification.

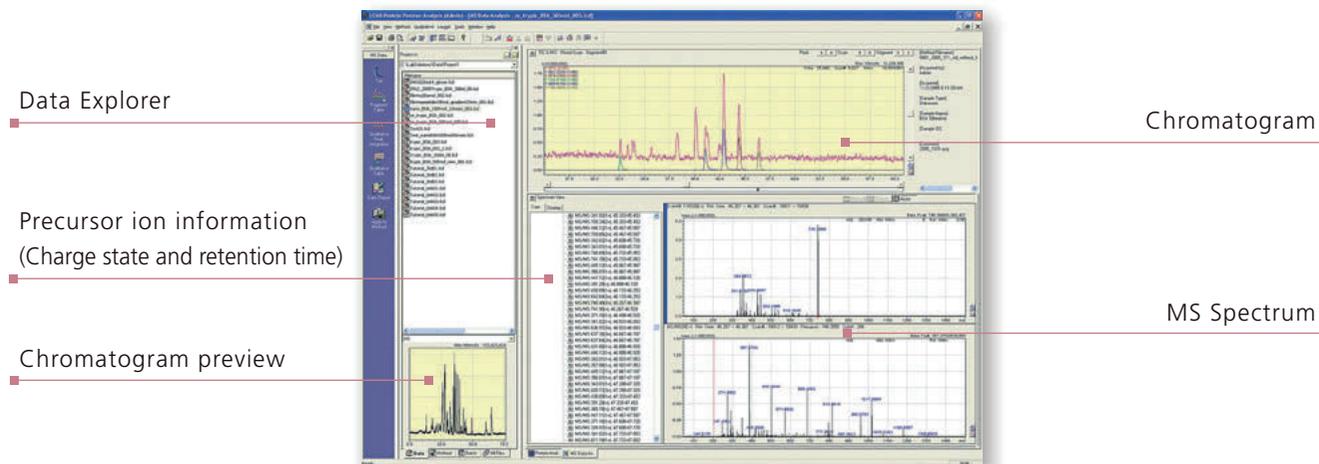
The identification results can be viewed directly from the Protein Layer software, enabling easy data management. Moreover, the parameters can be easily changed for follow-up searches.

The valuable time saved with the efficiency of this software makes it indispensable for protein analysis.

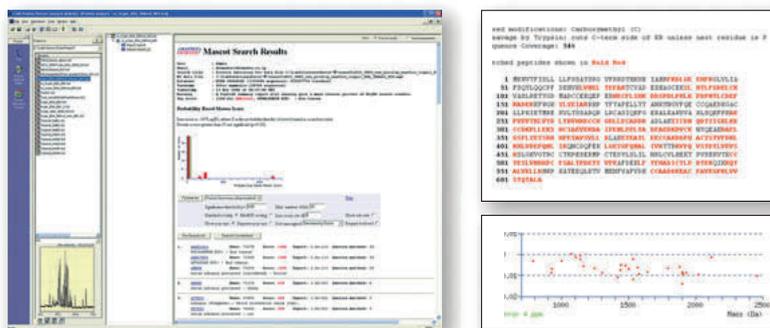


## A screen capture of the Protein Layer software

The MS data from the Protein Layer software data file and the Mascot search results can be viewed together.



Measurement Example (BSA Tryptic digest 50 fmol)

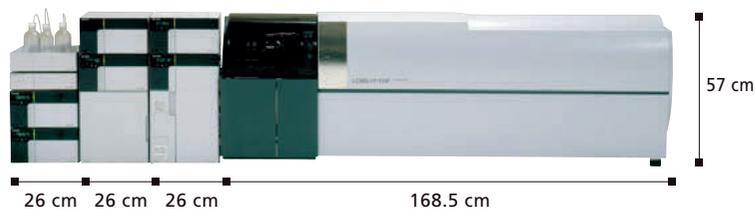


As a result of the Mascot search, BSA's score is 1180 and the No.1 ranking protein hit. Furthermore, all of the peptide masses are reported to be within the error range of 4 ppm or less in the identification results (data collected using an external standard).

This data exemplifies the high-quality measurement obtainable with the high-accuracy LCMS-IT-TOF.

# Prominence Series + LCMS-IT-TOF™

Installation View



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