SHIMADZU
Excellence in Science

Liquid Chromatograph Mass Spectrometer

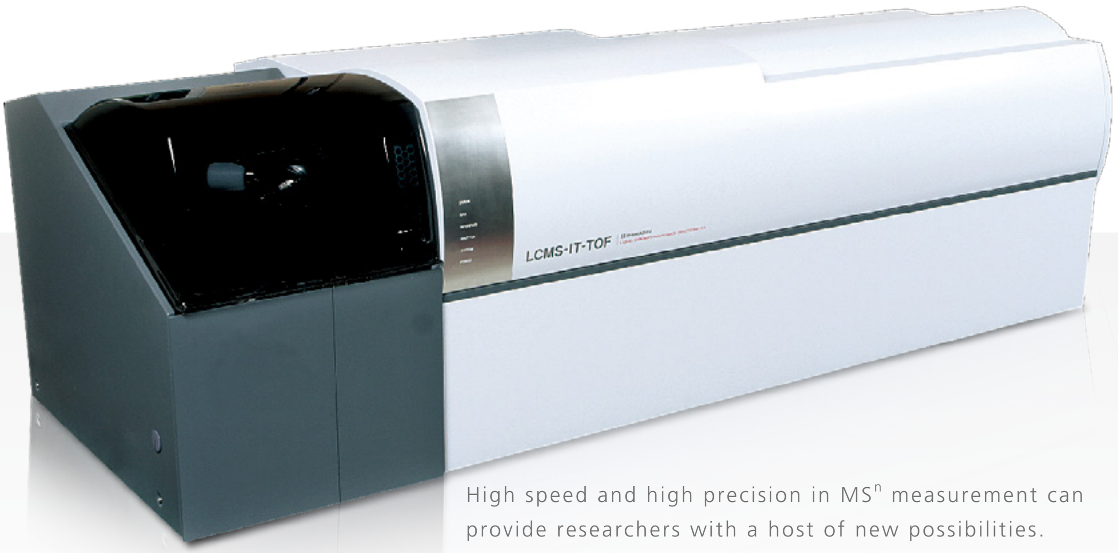
LCMS-IT-TOF

UFMS
ULTRA FAST MASS SPECTROMETRY

LCMS-IT-TOF

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

A significantly advanced MS detector enabling
LC/MS^n high-precision mass analysis



High speed and high precision in MS^n measurement can provide researchers with a host of new possibilities.

The LCMS-IT-TOF has been awarded the R&D 100 chosen by the U.S. technology information magazine *R&D*.

High Resolution & High Precision in MS^n measurement

The stable, high-resolution spectra achieved throughout a wide mass range and high-precision MS^n data are derived from the DSR: Dual-Stage Reflectron (*1) and the use of BIE: Ballistic Ion Extraction (*2). 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 (*3), allowing for the ions to be placed efficiently into the ion trap.

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*1 Patent US6384410, US6803564 and others
*2 Patent US6380666 and others
*3 Patent US6700116 and others

Speed Beyond Comparison

UFMS

ULTRA FAST MASS SPECTROMETRY

Original and Advanced Technology

What is the IT-TOF?

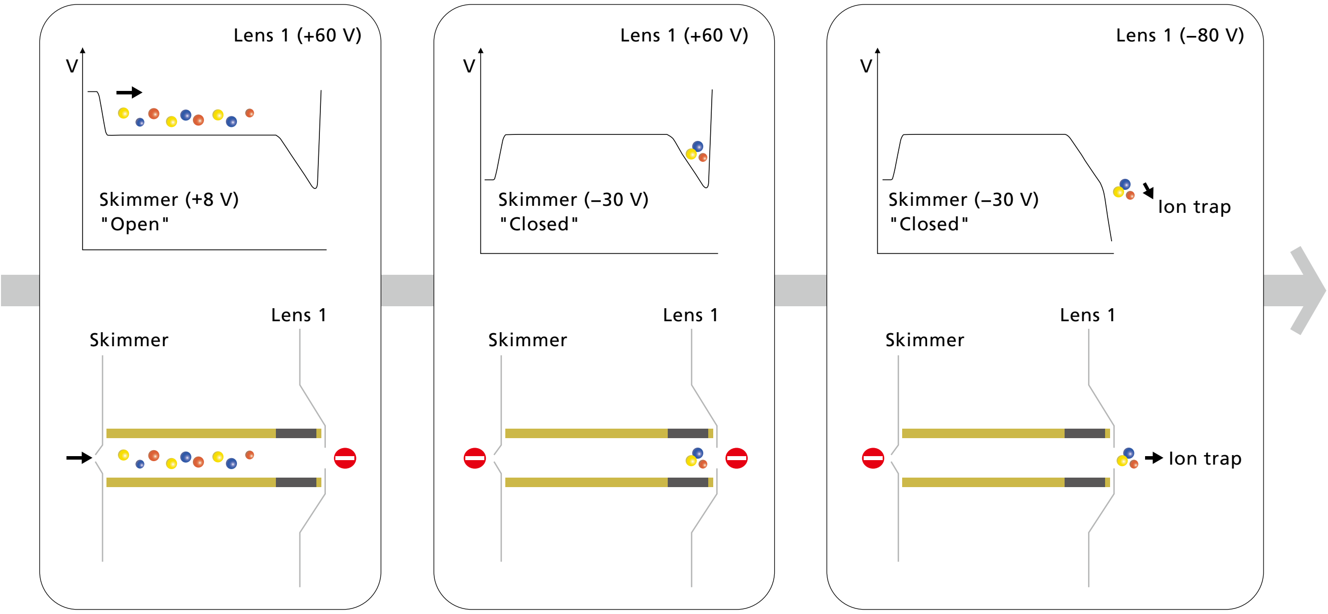
There are several types of LC/MS instruments in the market currently. The single quadrupole and triple quadrupole instruments are primarily used for quantitative analysis, while the ion trap, LC-TOF and Qq-TOF instruments are primarily used for qualitative analysis. Triple quadrupoles are excellent instruments 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^n; however, the mass accuracy and resolution, like with a triple quadrupole MS, are limited. On the other hand, the Qq-TOF features 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^3 or greater as their construction does not support MS^n.

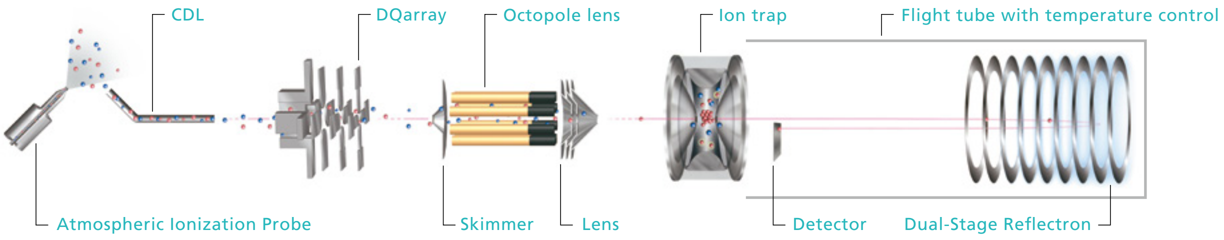
Shimadzu developed a new type of hybrid mass spectrometer, the LCMS-IT-TOF, which possesses both the MS^n ability of an ion trap and the excellent resolution and mass accuracy of a TOF.

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.



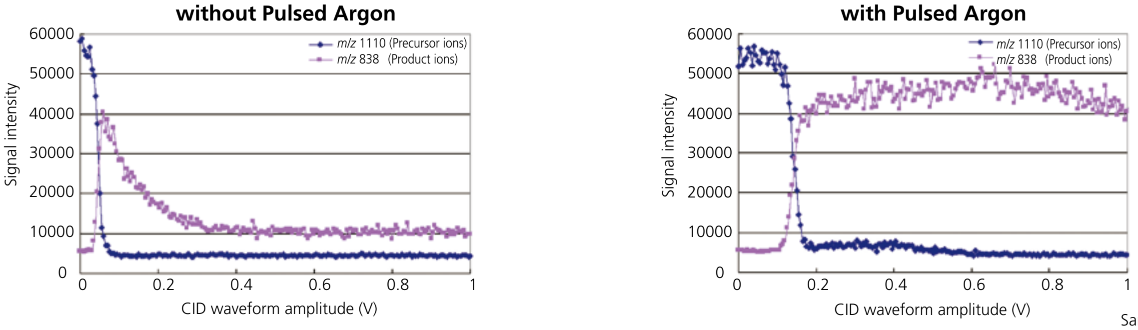


In the LCMS-IT-TOF, rather than simply combining the existing technologies of the QIT (Quadrupole Ion Trap) mass spectrometer and an oaTOF (Orthogonal Acceleration Time of Flight) mass spectrometer, the QIT and TOF are arranged linearly in a unique construction. This construction offsets the disadvantages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



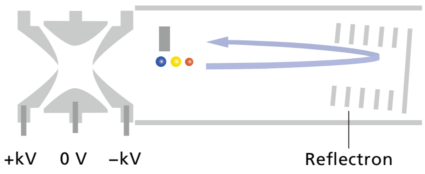
Sample: NaTFA

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 traditonal 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^n measurement of compounds having various *m/z*'s can be performed under a single set of CID conditions.

Addressing Sharp Chromatographic Peaks...

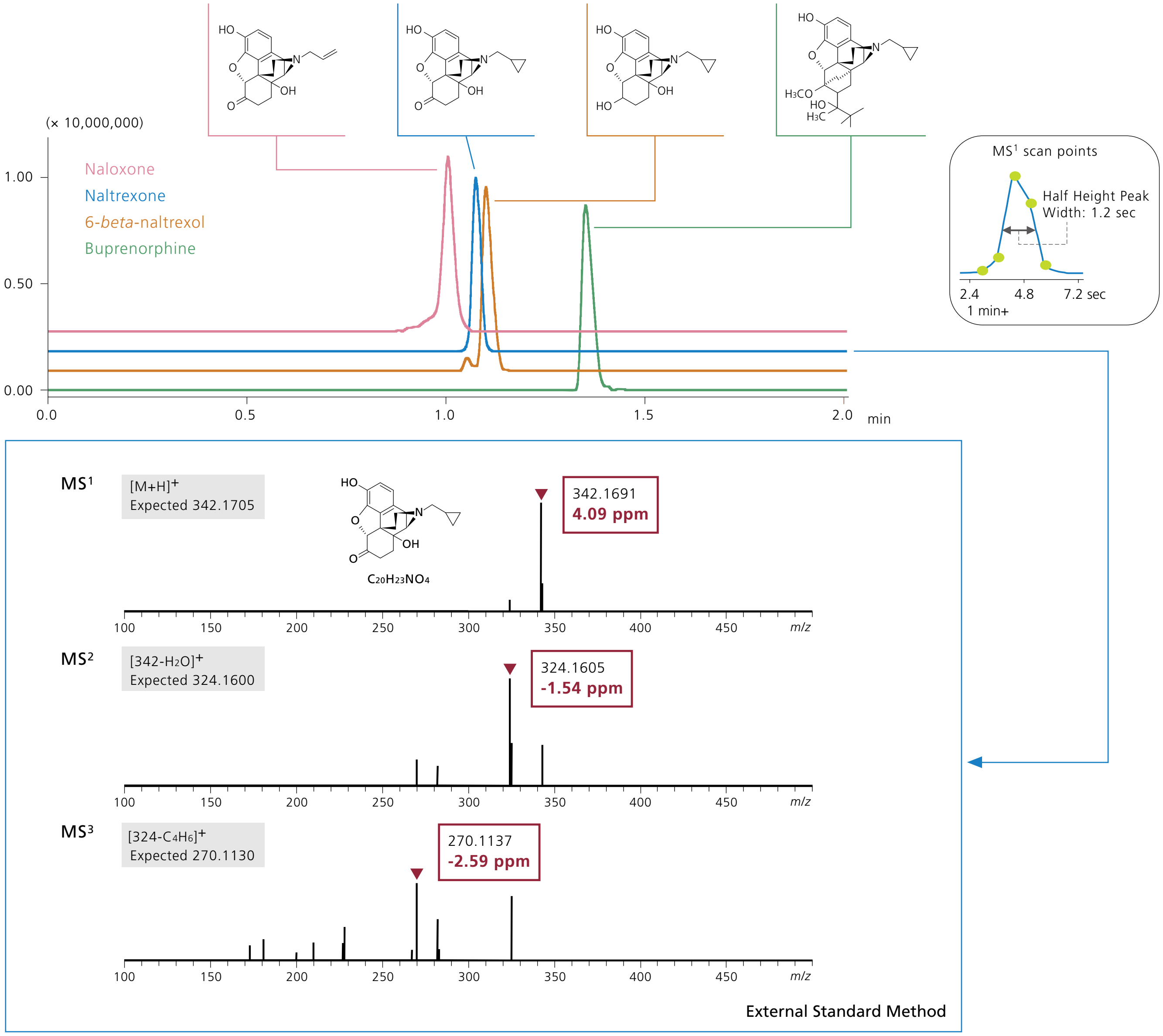
BIE [Ballistic Ion Extraction]

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.



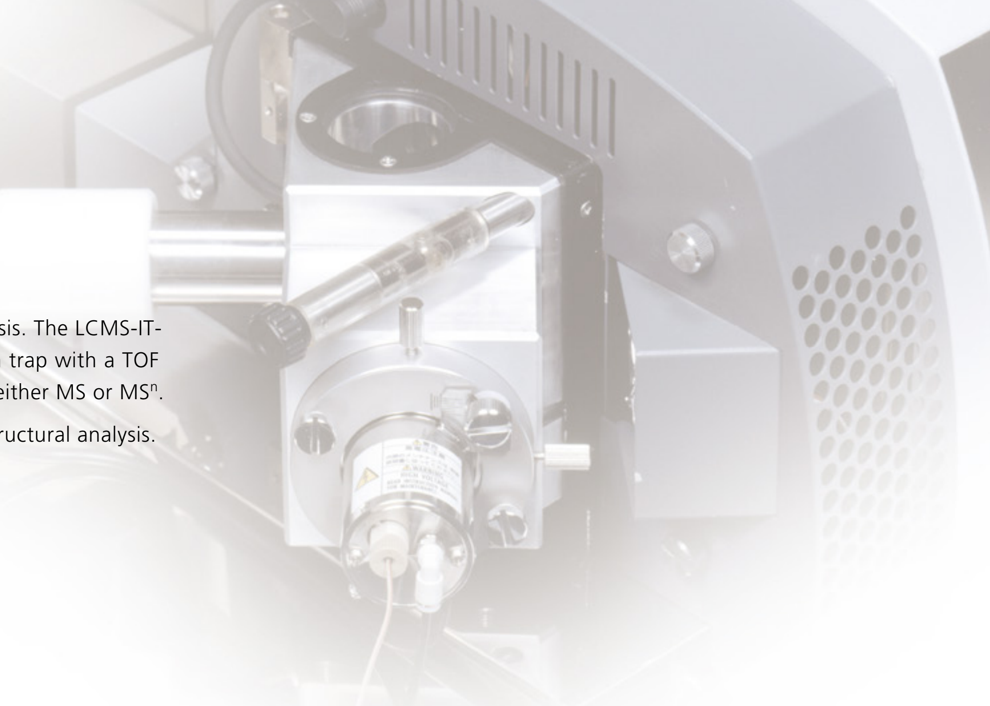
High-Speed and High-Accuracy MS^n Measurement

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



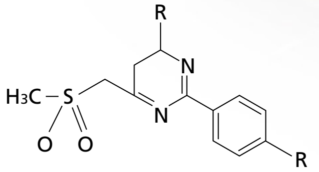
The data above shows the analysis of four compounds within 2.0 minutes. The Auto MS^n function makes it possible to obtain highly accurate MS^n data via an external standard, taking advantage of high-speed mass spectrum measurement performance.

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

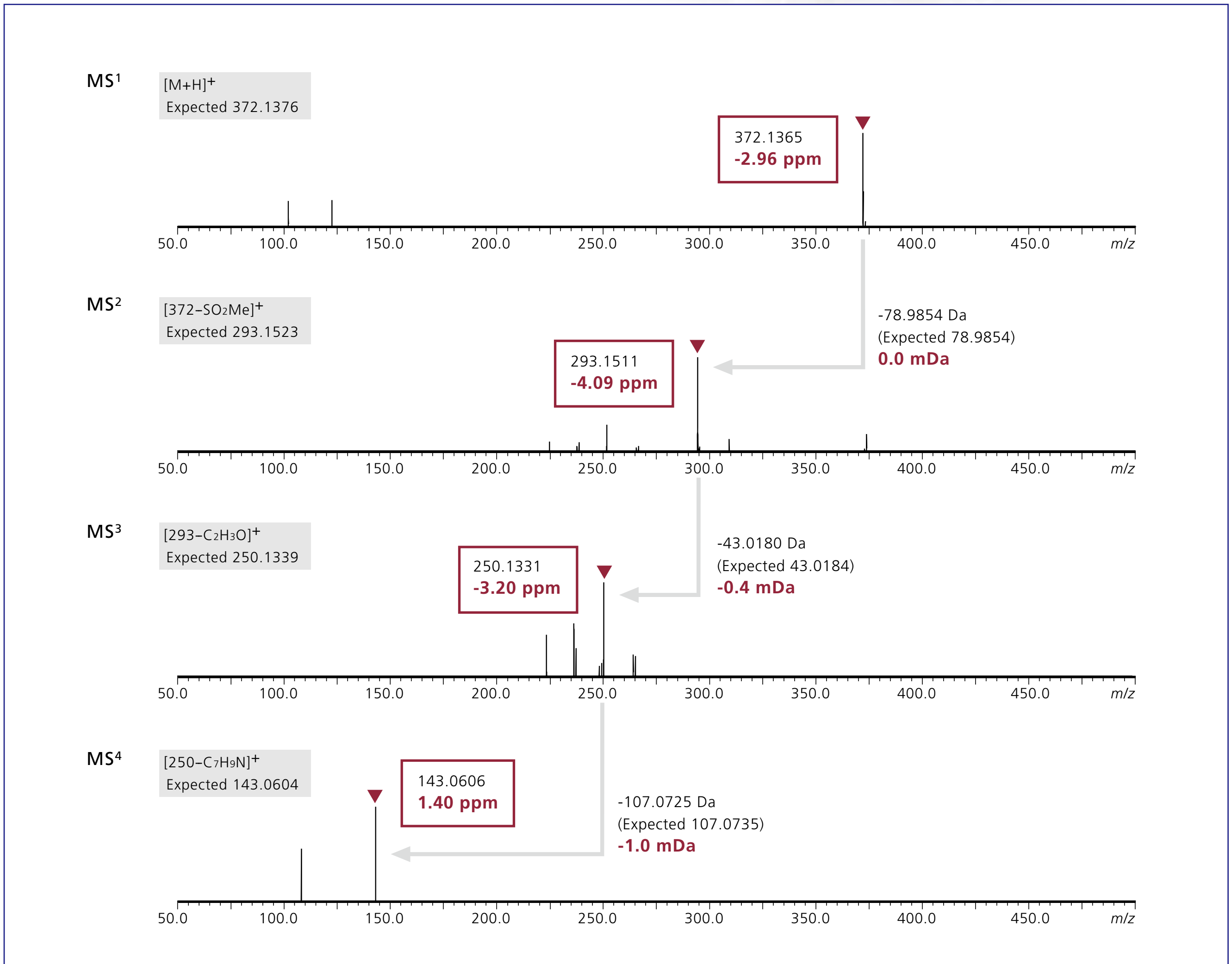


Ion fragmentation is very useful in structural analysis. The LCMS-IT-TOF has a unique design in that it connects an ion trap with a TOF and provides high-precision mass information for either MS or MS^n.

This makes it possible to perform highly reliable structural analysis.



$C_{19}H_{21}O_3N_3S$



External Standard Method

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

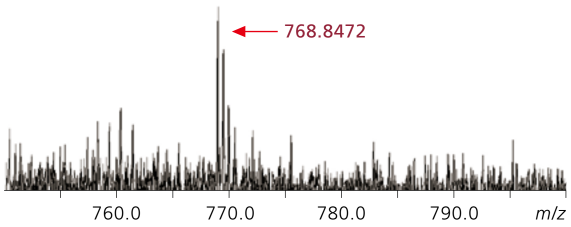
Sample provided by: AstraZeneca (UK) Dr. Richard Gallagher

Basic Performance Supporting High-Quality Data

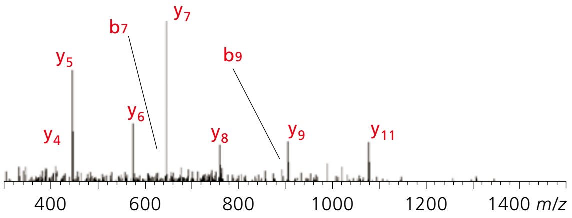
Compatibility of High Sensitivity and Mass Accuracy

100 attomol of Fibrinopeptide A

MS^1



MS^2



M.W. 1535.6852

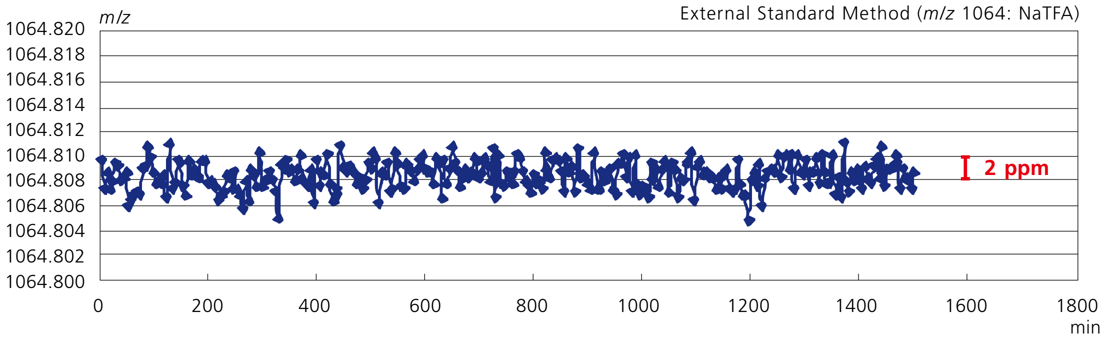
ADSGEGDFLA EGGGVR

Ion	Measured	Expected	Error (ppm)
$[M+2H]^{2+}$	768.8472	768.8506	–4.4

Ion	Measured	Expected	Error (ppm)
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

Extended Stability in Mass Accuracy

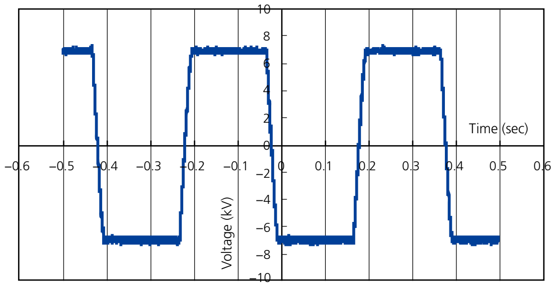
The high-accuracy temperature control mechanism of the flight tube, the localized ion due to BIE from the QIT, and the effective 10-bit high-speed transient recorder enable extremely stable mass accuracy using the external standard method. Due to labor-intensive work required when using an internal standard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

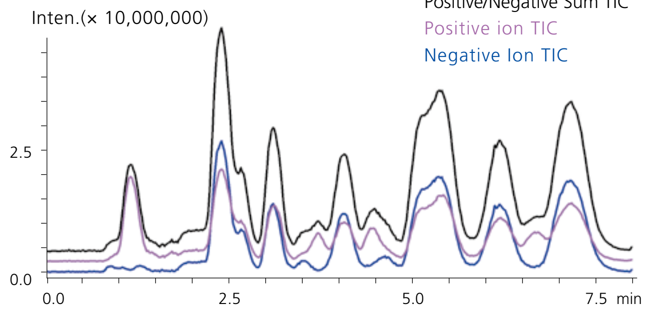
High-Speed Ion Polarity Switching can be especially useful when it cannot be judged whether samples will be detected as positive or negative ions. The LCMS-IT-TOF utilizes a newly developed, highly accurate and stable power supply as well as a newly developed high-voltage switch that allows for polarity 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.

Voltage change monitored with an oscilloscope



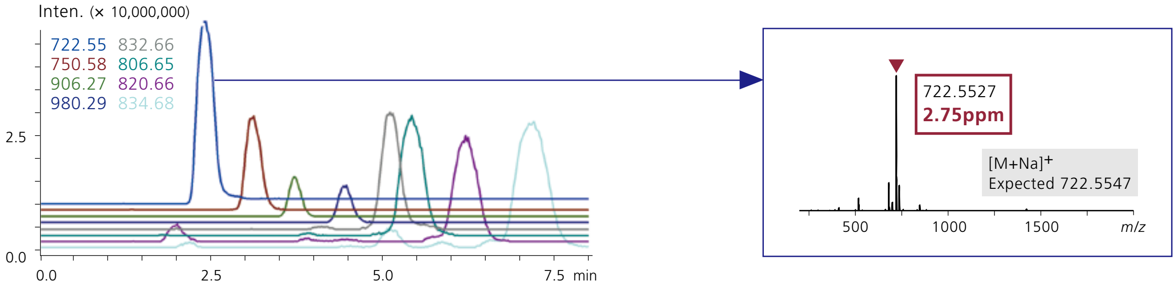
High-Speed Ion Polarity Switching Mode Measurement Example

Total Ion Chromatogram

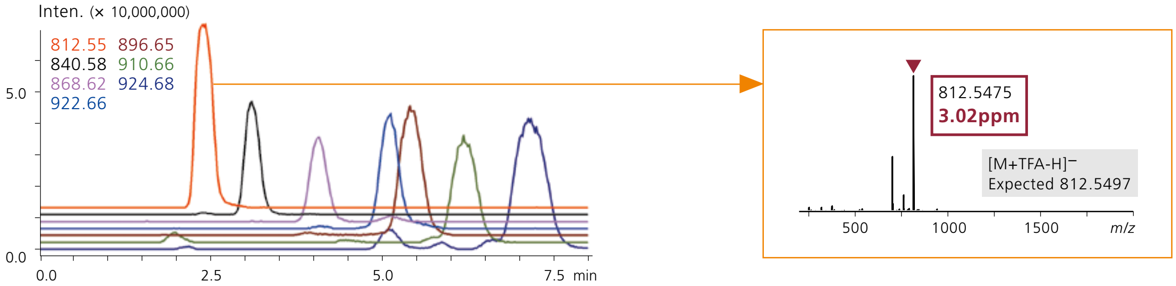


Sample	: glycosylceramide (C16,18,20,22,23,24:0-d18:1), (C24:1-d18:1)
Column	: Imtakt Cadenza CD-C18
Mobile phase	: 5 mM Acetic acid in methanol
Flow rate	: 0.2 mL/min

Positive Ion Mass Chromatogram



Negative Ion Mass Chromatogram



Provided by RIKEN

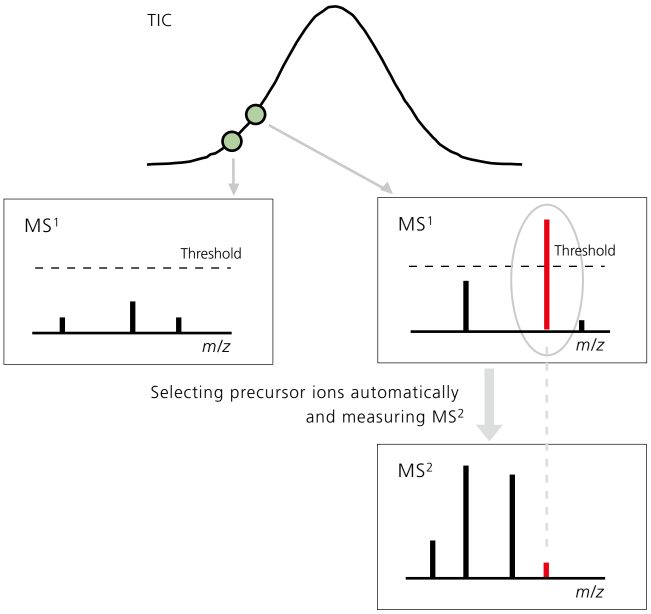
Intelligent Auto MS^n Function

What is the Auto MS^n 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

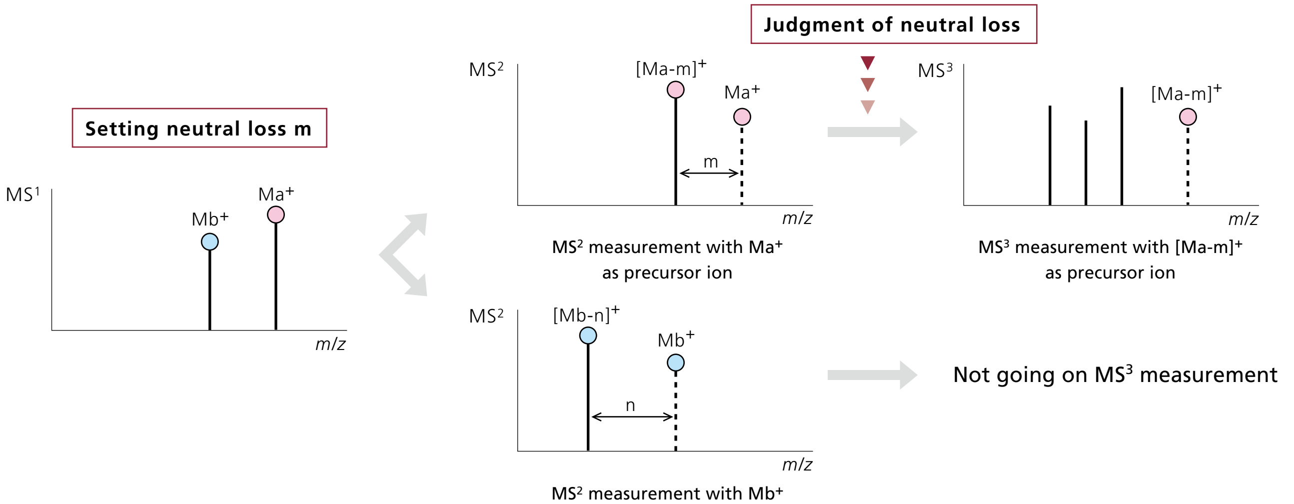
- Selection in the order of intensity or *m/z*
- Charge state filter
- Monoisotopic filter
- Exclusion ion (Automatic exclusion)
- Pair peak selection
- Preference Ion Selection
- Neutral Loss Survey



Selecting precursor ions automatically and measuring MS^2

Principles of Neutral Loss Survey

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



MS^2 measurement with Ma$^+$ as precursor ion

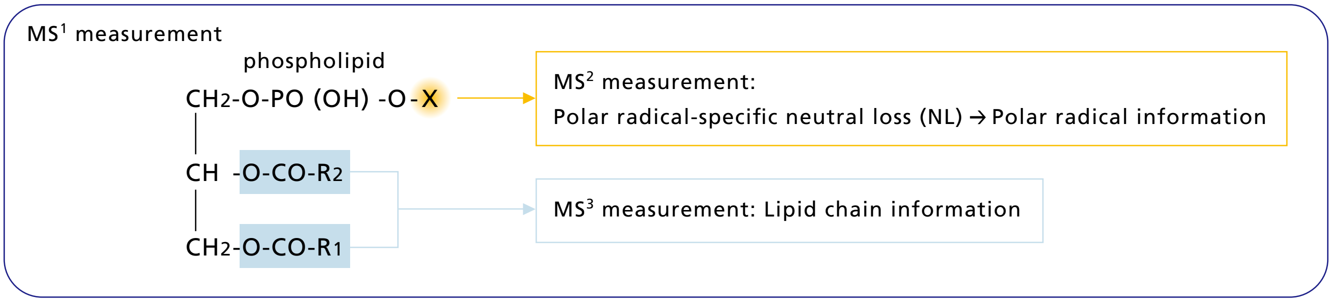
MS^3 measurement with [Ma-m]$^+$ as precursor ion

Not going on MS^3 measurement

MS^2 measurement with Mb$^+$ as precursor ion

Examples Highlighting the Neutral Loss Survey Function

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



MS^1 measurement

phospholipid

CH$_2$-O-PO (OH) -O-X

CH -O-CO-R$_2$

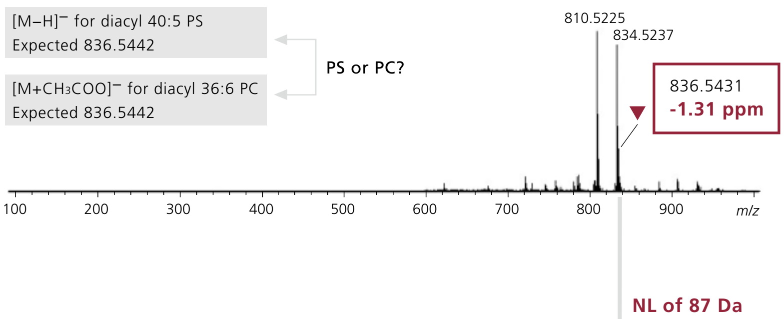
CH$_2$-O-CO-R$_1$

MS^2 measurement:
Polar radical-specific neutral loss (NL) → Polar radical information

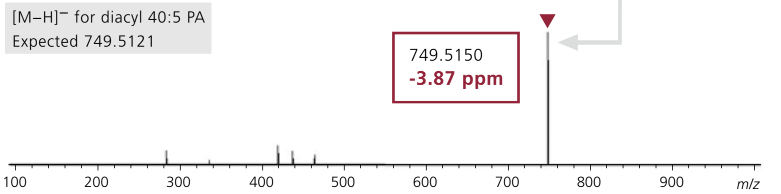
MS^3 measurement: Lipid chain information

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

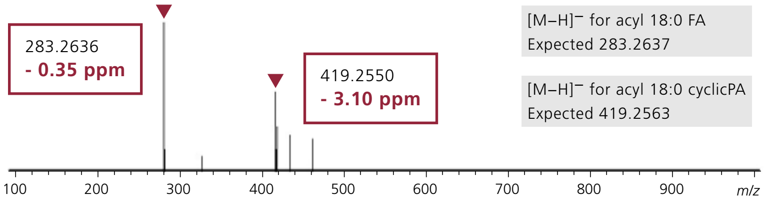
MS^1



MS^2 of *m/z* 836.5

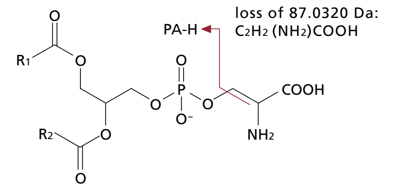


MS^3 of *m/z* 749.5



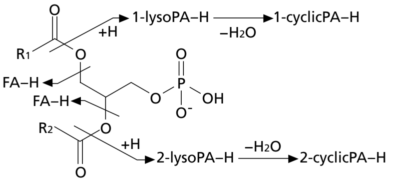
Using neutral loss survey, diacyl 18:0 – 22:5 PCs in the lipid mixture were easily identified. Mass accuracy of less than 5 ppm was obtained in MS^1 through MS^3 using an external standard.

PC: Phosphatidilcholine
PS: Phosphatidilserine
PA: Phosphatidic acid
FA: Fatty acid



Detection of PS-specific NL: Polar radical information

Performing MS^3 measurement of precursor ions at *m/z* 749 by neutral loss survey



MS^3 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)

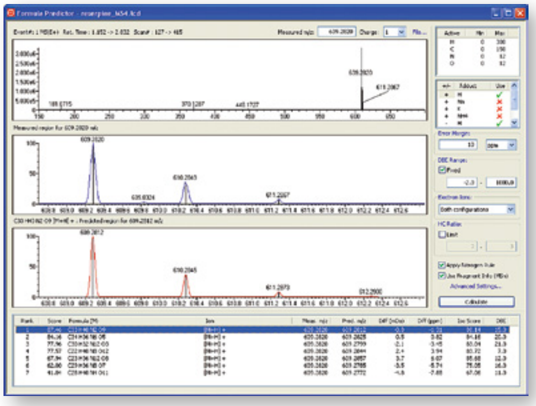
Optional Software for Accelerating Data Analysis

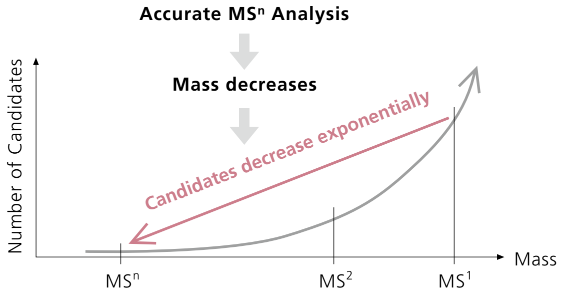
Formula Predictor

Effectiveness of Accurate MS^n

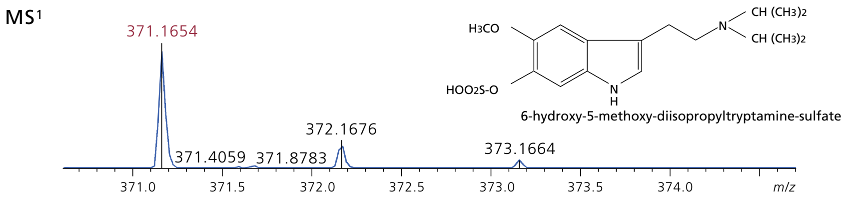
In composition prediction, target constituents having a small mass and high mass accuracy associated with their measured values, provide fewer numbers of candidates and greater prediction reliability.

When using MS^2 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.

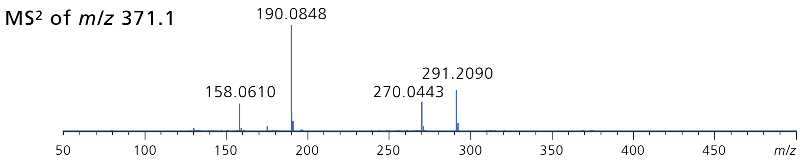




$C_{17}H_{26}N_2O_5S+H^+$ (*m/z* 371.1641) Composition Prediction Example



6-hydroxy-5-methoxy-diisopropyltryptamine-sulfate



(1) Composition Calculated from Mass		(2) Using Isotopic Pattern		(3) MS^n Spectral Filtering	
#	Formula	#	Formula	#	Formula
1	C18 H22 N6 O S	**1**	**C17 H26 N2 O5 S**	**1**	**C17 H26 N2 O5 S**
2	C10 H18 N12 O4	2	C10 H18 N12 O4	2	C6 H26 N8 O8 S
3	C25 H22 O3	3	C18 H22 N6 O S		
4	C13 H26 N2 O10	4	C9 H22 N8 O8		
5	**C17 H26 N2 O5 S**	5	C13 H26 N2 O10		
6	C9 H22 N8 O8	6	C25 H22 O3		
7	C6 H26 N8 O8 S	7	C6 H26 N8 O8 S		
8	C14 H22 N6 O6	8	C8 H26 N4 O12		
9	C22 H26 O3 S	9	C14 H22 N6 O6		
10	C8 H26 N4 O12	10	C22 H26 O3 S		
11	C7 H22 N12 O4 S	11	C21 H18 N6 O		
12	C21 H18 N6 O	12	C7 H22 N12 O4 S		

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

The correct composition is $C_{17}H_{26}N_2O_5S+H^+$ (*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^n Spectral Filtering

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

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

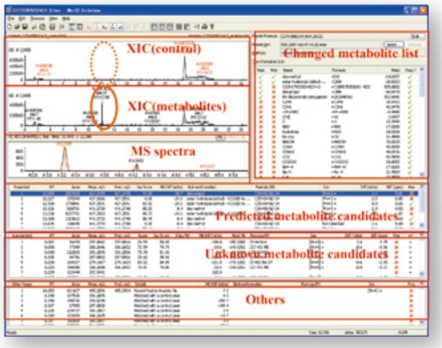
MetID Solution

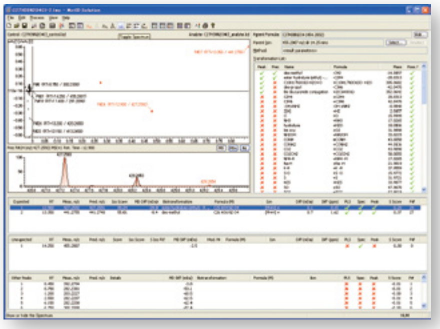
Comprehensive Metabolite Detection and Structure Prediction Using Accurate Mass Information from HPLC and Online MS^n

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^n 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.

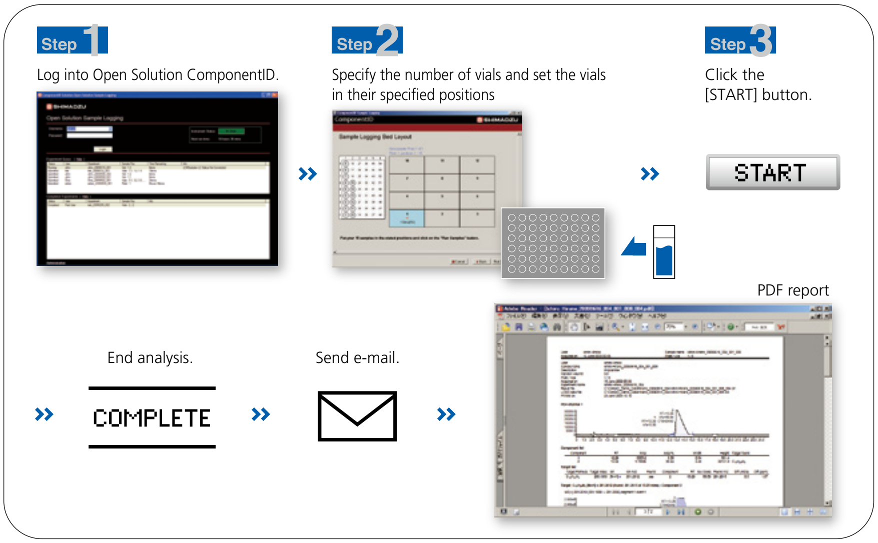




Open Solution ComponentID

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.



Step 1
Log into Open Solution ComponentID.

»

Step 2
Specify the number of vials and set the vials in their specified positions

»

Step 3
Click the [START] button.

» End analysis.

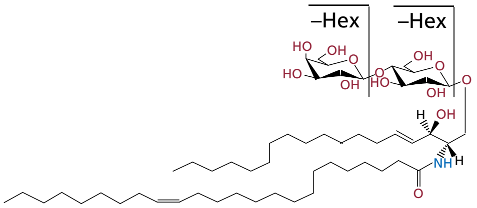
» Send e-mail.

» PDF report

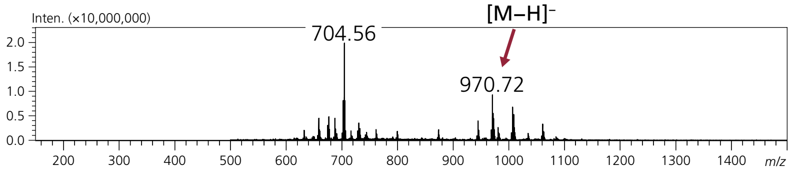
MS Spectra Library for Glycosphingolipid

Powerfully Supports the Structural Prediction for Glycolipids

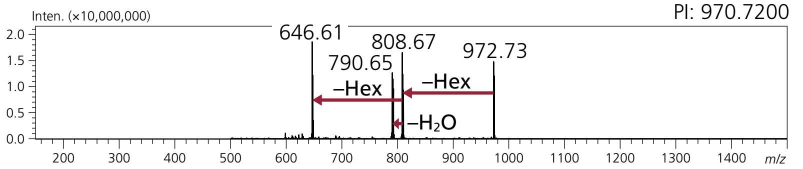
Glycolipid is a collective term for compounds with a sugar chain bonded to a lipid. It has attracted attention as a target of research in various applications including drug discovery and the development of cosmetics. The main content of this library is gangliosides. It contains the data of 258 species including ceramides and acidic glycolipids. This library includes precise mass data acquired by extracting and purifying actual samples using the LCMS-IT-TOF MS^n analysis function as well as up to MS^4 spectra. It also contains data of many glycolipids for which standard products are not commercially available. Using the MS Spectra Library for Glycosphingolipid enables identification of the structure of lipid moiety in addition to the sugar chain structure of trace amounts of glycolipids present in living bodies.



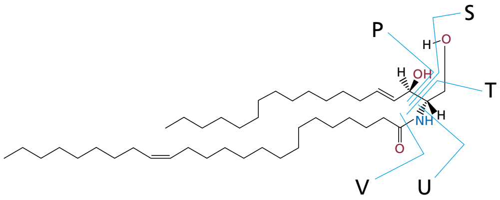
LacCer (d18:1-24:1)
Exact Mass: 971.7273

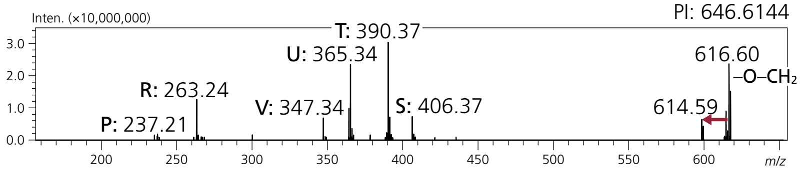


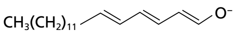
MS Spectrum



MS/MS Spectrum Precursor *m/z* 970.72







R = *m/z* 263

MS/MS/MS Spectrum Precursor *m/z* 646.61

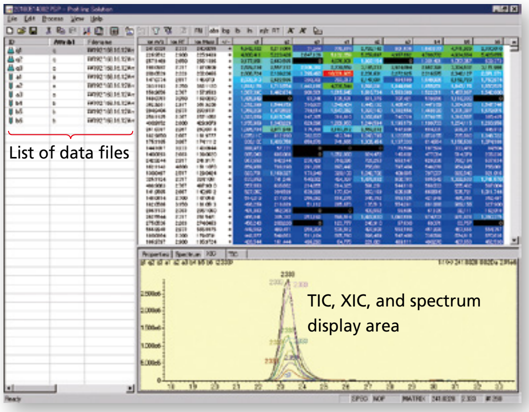
Profiling Analysis Software Profiling Solution

Creates Data Array by Extracting Peaks from Multiple Data Files

Creating a table from a huge amount of data and finding variations (differences) between data are critical steps in metabolomics and differential analysis.

Profiling Solution can automatically detect peaks in many data files acquired from an LC-MS or GC-MS system and create a table using the data. This table can be analyzed easily in various methods using commercial statistical software.

Table of peaks extracted from each data file



List of data files

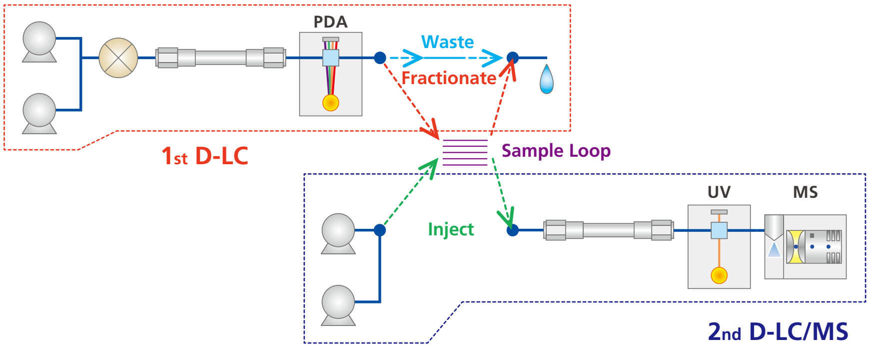
TIC, XIC, and spectrum display area

More Efficient Identification of Impurities

Trap-free 2D LC/MS Impurity Identification System

Online Switching between a Non-volatile Mobile Phase and a Volatile Mobile Phase

Non-volatile mobile phases commonly used for HPLC are not suitable for LC/MS. Therefore, the conditions need to be changed to volatile mobile phase conditions when estimating the structure of impurities detected under HPLC conditions. This system performs LC/MS measurements on impurities through the combination of 2DLC and LCMS-IT-TOF while switching between a non-volatile mobile phase and a volatile mobile phase online. This significantly reduces the time required for volatile mobile phase method development and enables more efficient identification of impurities.

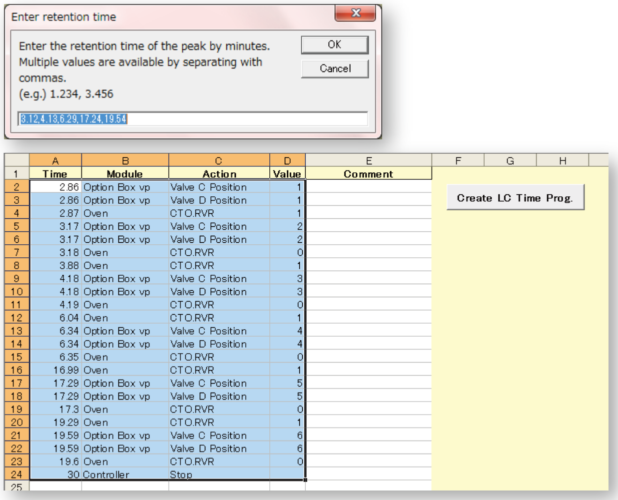


System Flow Diagram

Supports Processes from Acquisition to Analysis of Impurities

Using support tools enables the easy creation of an optimum time program and batch schedule for acquiring data on multiple impurities and their respective blank data.

After data acquisition, using the data browser function provided with LCMSsolution makes it easy to compare the sample data and blank data, enhancing the efficiency of the impurity identification process.

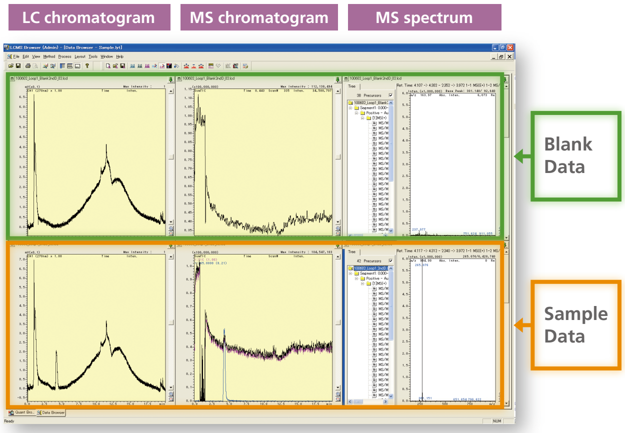


Enter the retention time of the peak by minutes.
Multiple values are available by separating with commas.
(e.g.) 1.234, 3.456

3.12,4.13,6.29,17.24,19.54

	A	B	C	D	E	F	G	H
1	Time	Module	Action	Value	Comment			
2	2.86	Option Box vp	Valve C Position	1				
3	2.86	Option Box vp	Valve D Position	1				
4	2.87	Oven	CTO.RVR	1				
5	3.17	Option Box vp	Valve C Position	2				
6	3.17	Option Box vp	Valve D Position	2				
7	3.18	Oven	CTO.RVR	0				
8	3.88	Oven	CTO.RVR	1				
9	4.18	Option Box vp	Valve C Position	3				
10	4.18	Option Box vp	Valve D Position	3				
11	4.19	Oven	CTO.RVR	0				
12	6.04	Oven	CTO.RVR	1				
13	6.34	Option Box vp	Valve C Position	4				
14	6.34	Option Box vp	Valve D Position	4				
15	6.35	Oven	CTO.RVR	0				
16	16.99	Oven	CTO.RVR	1				
17	17.29	Option Box vp	Valve C Position	5				
18	17.29	Option Box vp	Valve D Position	5				
19	17.3	Oven	CTO.RVR	0				
20	19.29	Oven	CTO.RVR	1				
21	19.59	Option Box vp	Valve C Position	6				
22	19.59	Option Box vp	Valve D Position	6				
23	19.6	Oven	CTO.RVR	0				
24	30	Controller	Stop					
25								

The optimum valve sequence can be constructed simply by entering the retention time for impurity peaks. Paste it in a time program in LCMSsolution.



Impurity peaks can be found at a glance by displaying the blank and sample data on the top and bottom. This also helps the identification proceed smoothly.

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