Access to **your** success

⊕ **SHIMADZU**

LCMS-IT-TOF®

Shimadzu
High-Speed Liquid Chromatograph Mass Spectrometer
LCMS-IT-TOF®

LCMS-IT-TOF®,

a significantly advanced MS detector enabling

LC/MS^n high-precision mass analysis

LCMS-IT-TOF®

Shimadzu

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

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

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.

Contents

(*1) Patent US6384410, US6803564 and others (*2) Patent US6380666 and others
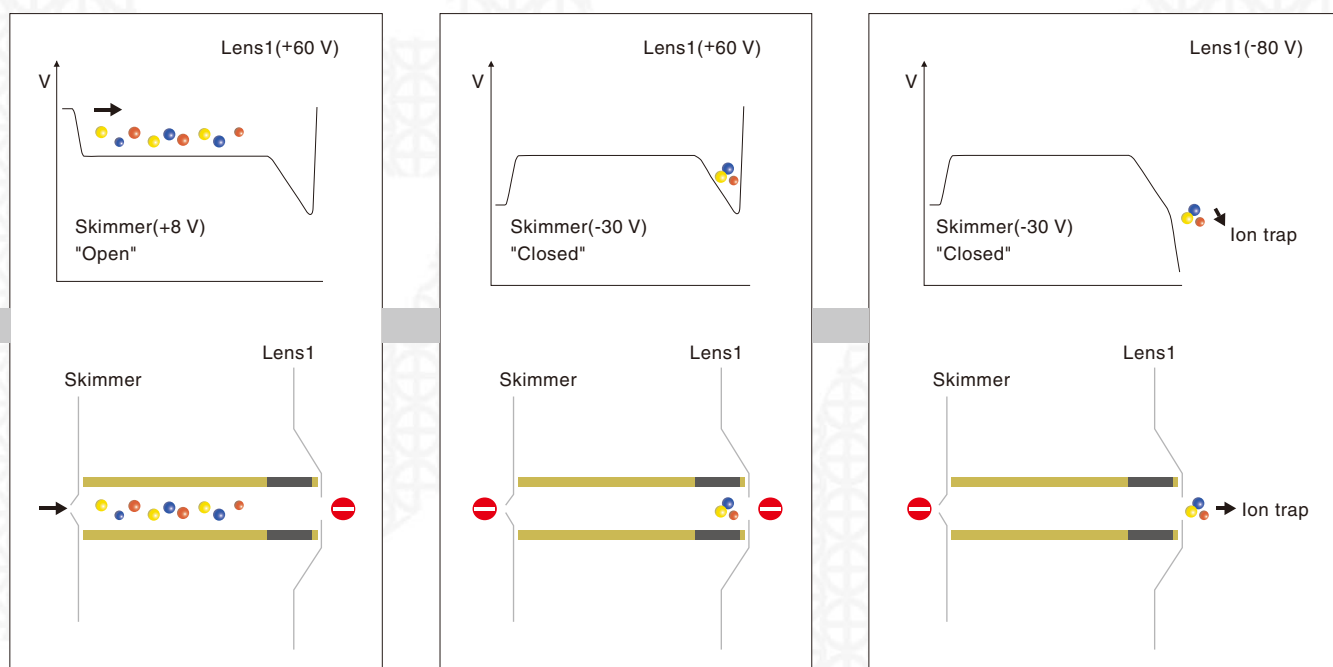(*3) Patent US6700116 and others
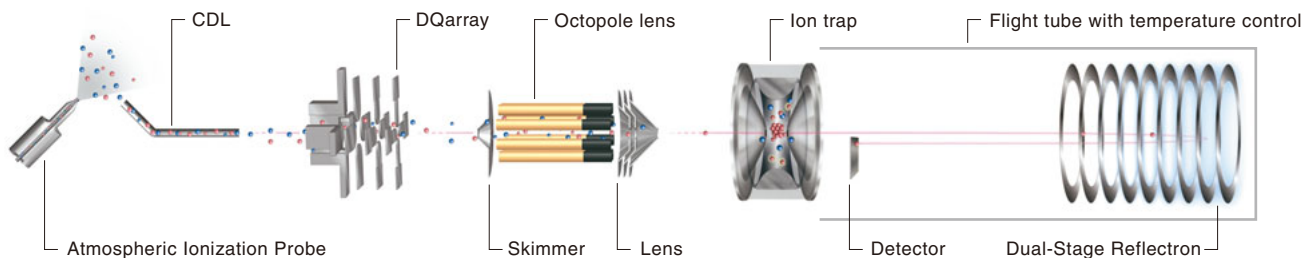
Original and Advanced Technology

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.

Lens1(+60 V)
V
Skimmer(+8 V) "Open"
Skimmer   Lens1

Lens1(+60 V)
V
Skimmer(-30 V) "Closed"
Skimmer   Lens1

Lens1(-80 V)
V
Skimmer(-30 V) "Closed"   Ion trap
Skimmer   Lens1   Ion trap

CDL — DQarray — Octopole lens — Ion trap — Flight tube with temperature control

Atmospheric Ionization Probe — Skimmer — Lens — Detector — Dual-Stage Reflectron

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.
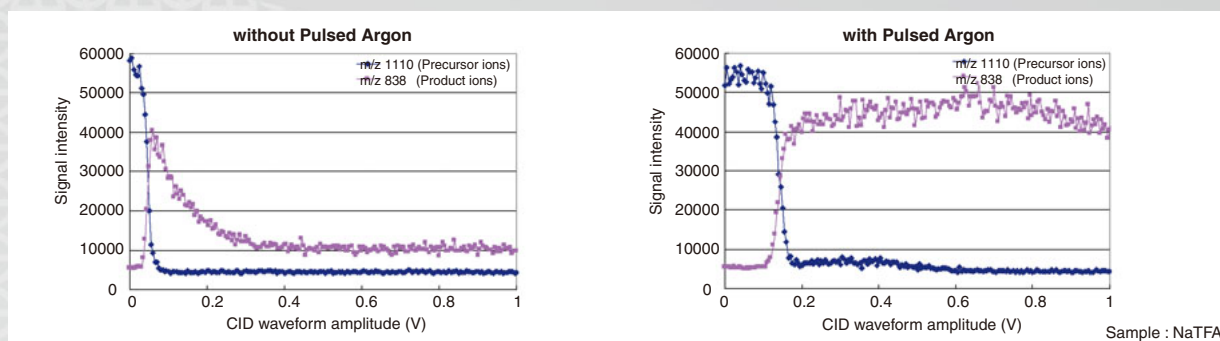

Without pulsed Ar introduction, product ion intensity noticeably decreases as the CID energy is increased. On the other hand, with pulsed Ar introduction, the product ion intensity remains unchanged even as the CID energy increases. In the case of traditonal ion traps, it is necessary to adjust the CID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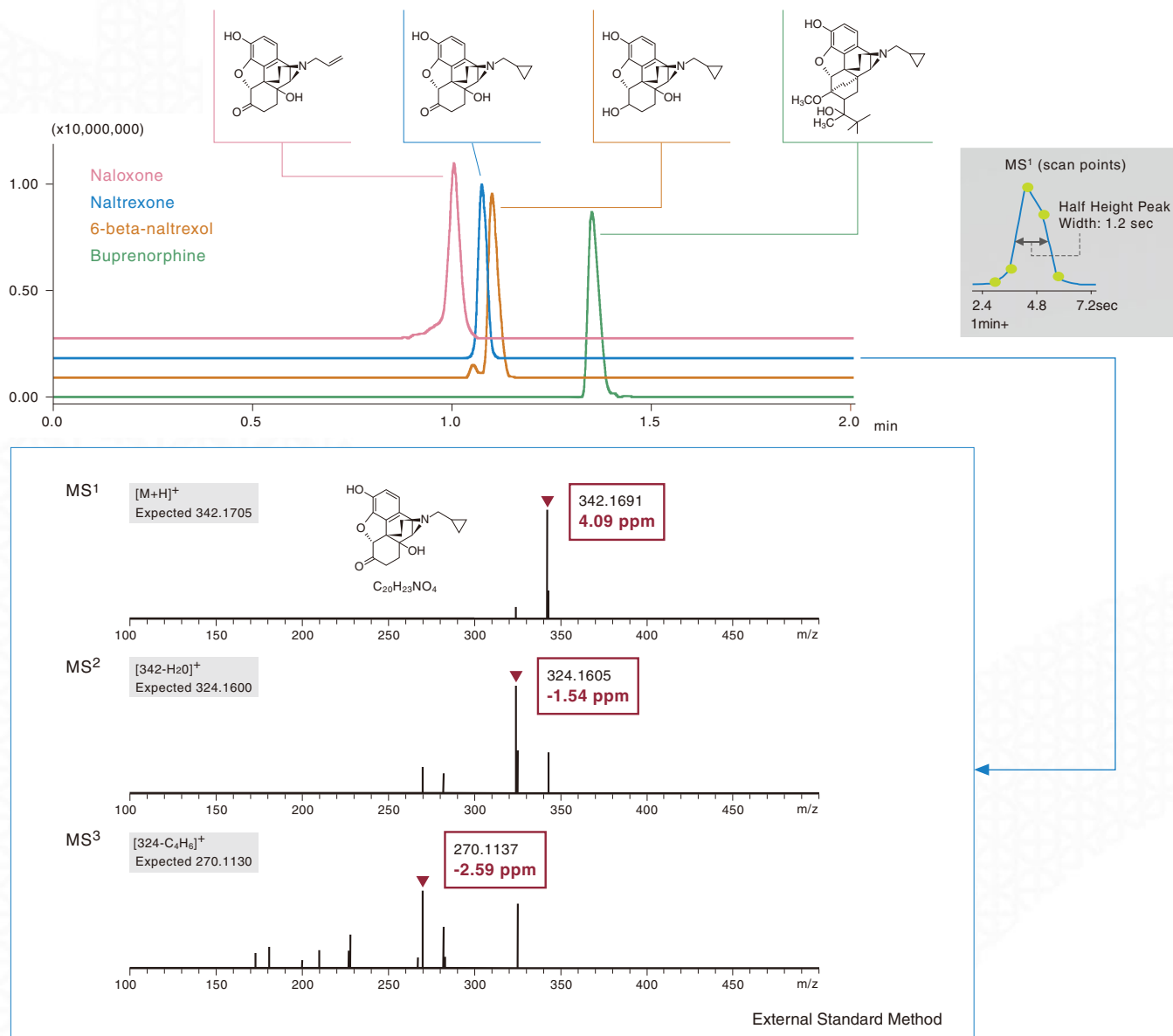
BIE [Ballistic Ion Extraction]

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.

+kV   O V   -kV   Reflectron
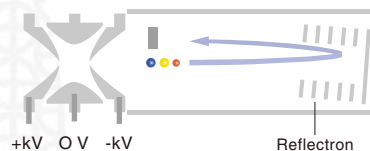
High-Speed, High-Accuracy MS^n Me

High-Speed, High-Accuracy MS^n Measurement Example

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.
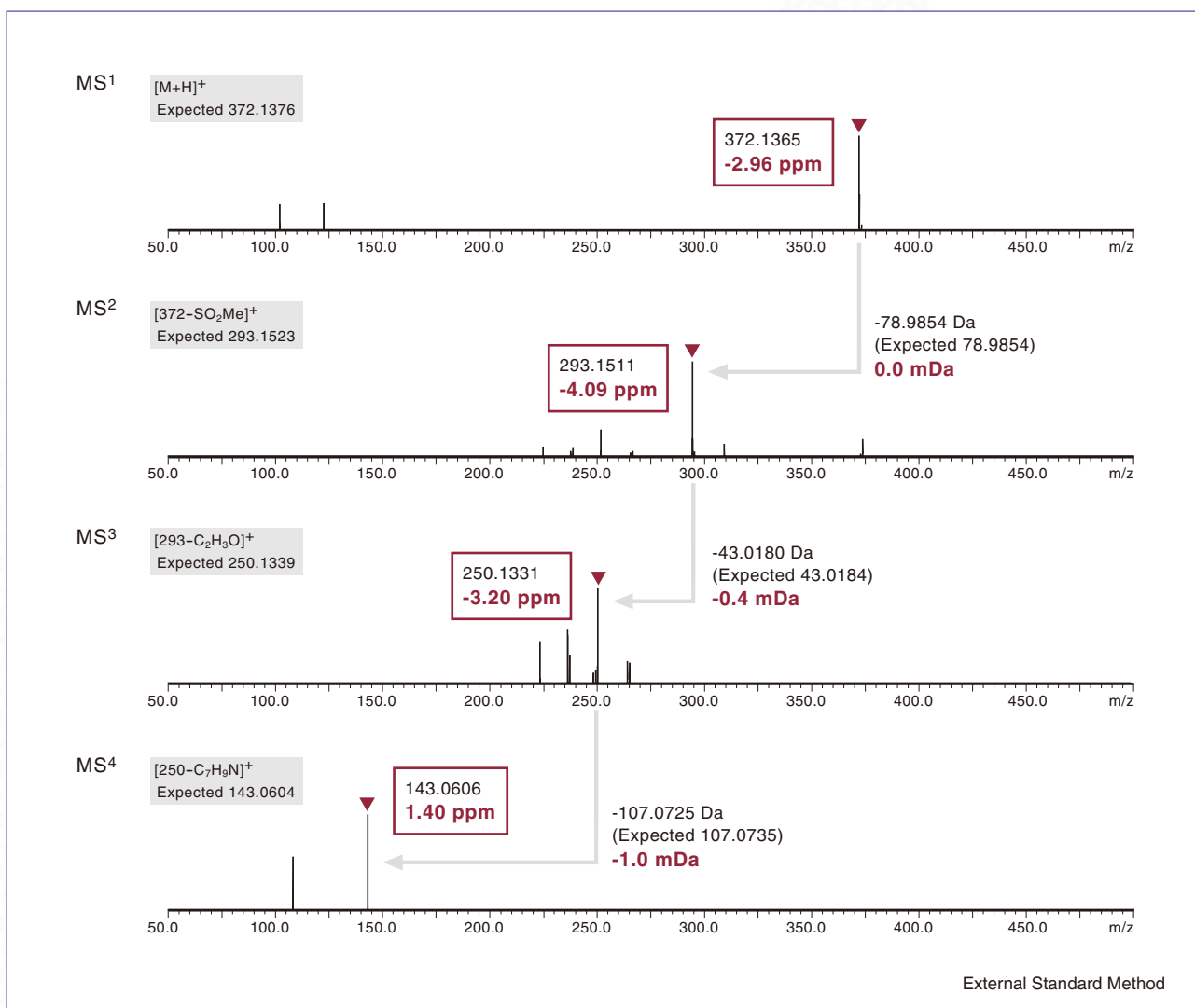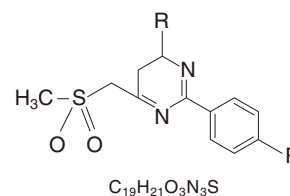

(x10,000,000)

Naloxone
Naltrexone
6-beta-naltrexol
Buprenorphine

MS^1 (scan points)

Half Height Peak Width: 1.2 sec

2.4   4.8   7.2sec
1min+

MS^1 [M+H]$^+$ Expected 342.1705

C$_{20}$H$_{23}$NO$_4$

342.1691
4.09 ppm

MS^2 [342-H$_2$0]$^+$ Expected 324.1600

324.1605
-1.54 ppm

MS^3 [324-C$_4$H$_6$]$^+$ Expected 270.1130

270.1137
-2.59 ppm

External Standard Method

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 x 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	: 5μL (1ng/μL)
Column oven temperature	: 50°C

asurement

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.

$C_{19}H_{21}O_3N_3S$

MS^1

[M+H]$^+$
Expected 372.1376

372.1365
-2.96 ppm

50.0 100.0 150.0 200.0 250.0 300.0 350.0 400.0 450.0 m/z

MS^2

[372–SO$_2$Me]$^+$
Expected 293.1523

293.1511
-4.09 ppm

-78.9854 Da
(Expected 78.9854)
0.0 mDa

50.0 100.0 150.0 200.0 250.0 300.0 350.0 400.0 450.0 m/z

MS^3

[293–C$_2$H$_3$O]$^+$
Expected 250.1339

250.1331
-3.20 ppm

-43.0180 Da
(Expected 43.0184)
-0.4 mDa

50.0 100.0 150.0 200.0 250.0 300.0 350.0 400.0 450.0 m/z

MS^4

[250–C$_7$H$_9$N]$^+$
Expected 143.0604

143.0606
1.40 ppm

-107.0725 Da
(Expected 107.0735)
-1.0 mDa

50.0 100.0 150.0 200.0 250.0 300.0 350.0 400.0 450.0 m/z
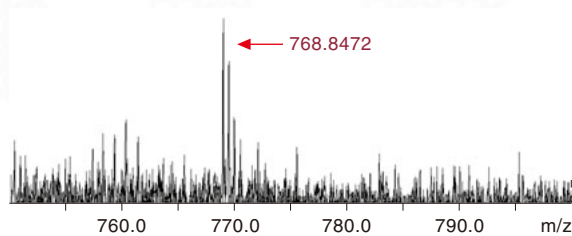
External Standard Method

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
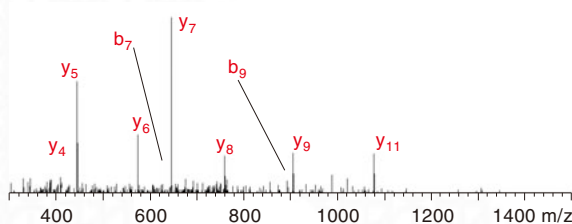
Basic Performance Supporting High

Compatibility of High Sensitivity and Mass Accuracy

100 attomol of Fibrinopeptide A

MS¹

768.8472

760.0   770.0   780.0   790.0   m/z

MS²

y5
b7
y7
b9
y4
y6
y8
y9
y11

400   600   800   1000   1200   1400 m/z

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.

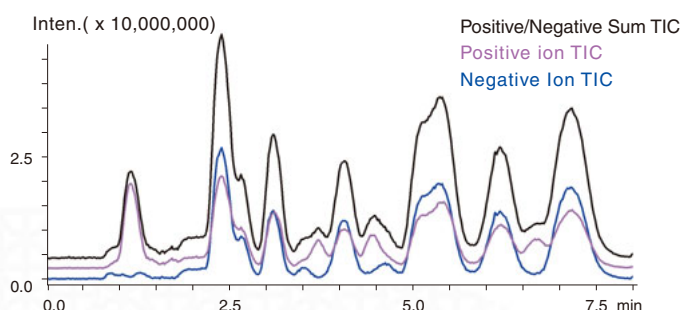External Standard Method (m/z 1064: NaTFA)

2ppm
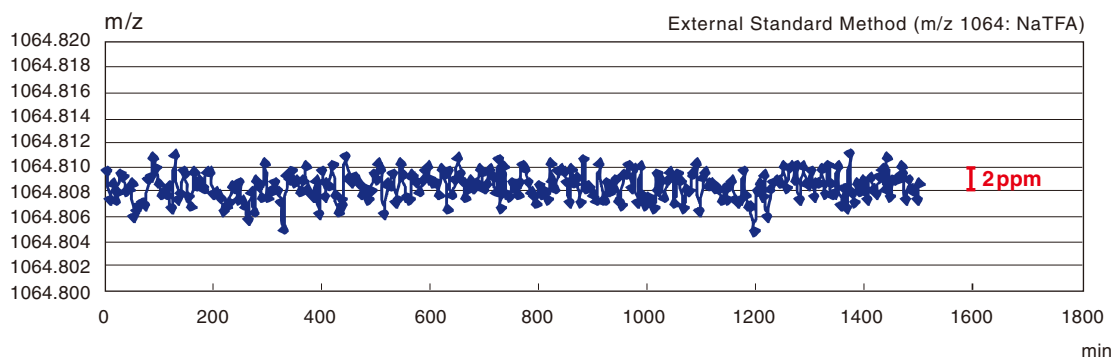
-Quality Data

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.
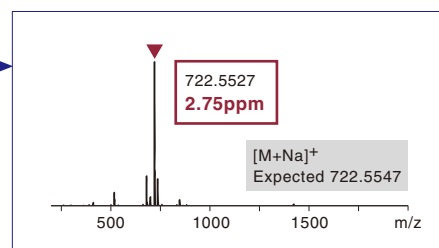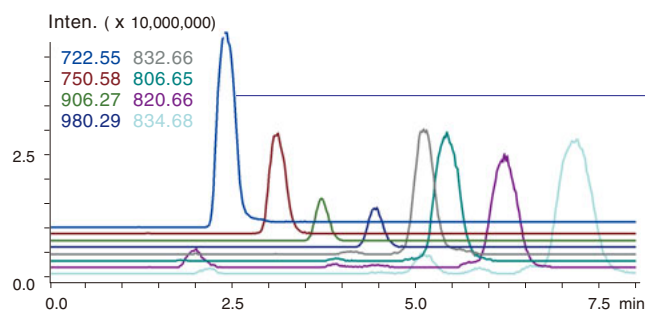
Voltage change monitored with an oscilloscope
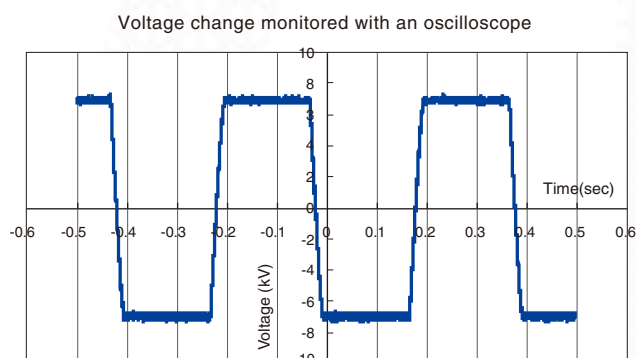
High-Speed Ion Polarity Switching Mode Measurement Example

Total Ion Chromatogram

Positive/Negative Sum TIC
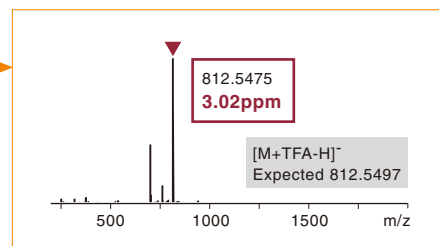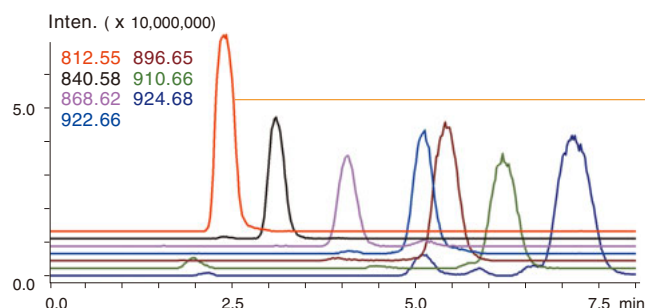Positive ion TIC
Negative Ion TIC

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
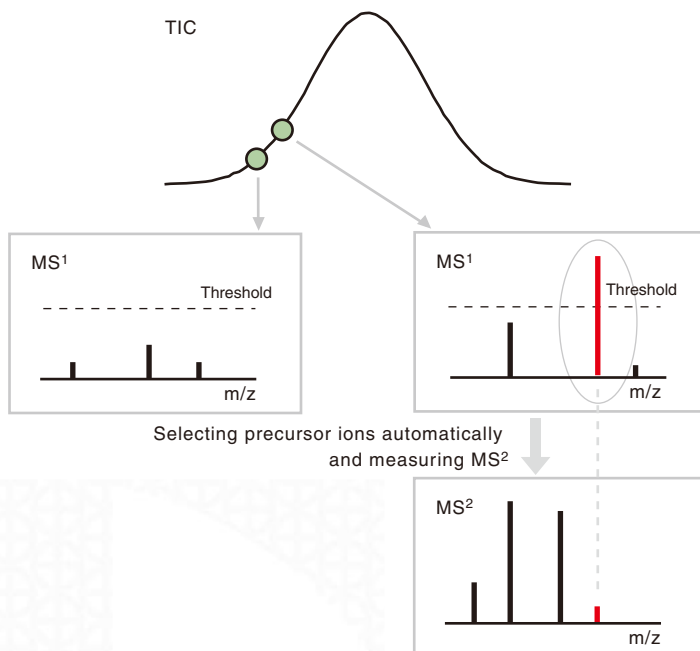Flow rate : 0.2 mL/min

Positive Ion Mass Chromatogram

Inten. (x 10,000,000)

722.55 832.66
750.58 806.65
906.27 820.66
980.29 834.68

722.5527
2.75ppm

$[M+Na]^+$
Expected 722.5547

Negative Ion Mass Chromatogram

Inten. (x 10,000,000)

812.55 896.65
840.58 910.66
868.62 924.68
922.66

812.5475
3.02ppm

$[M+TFA-H]^-$
Expected 812.5497

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.
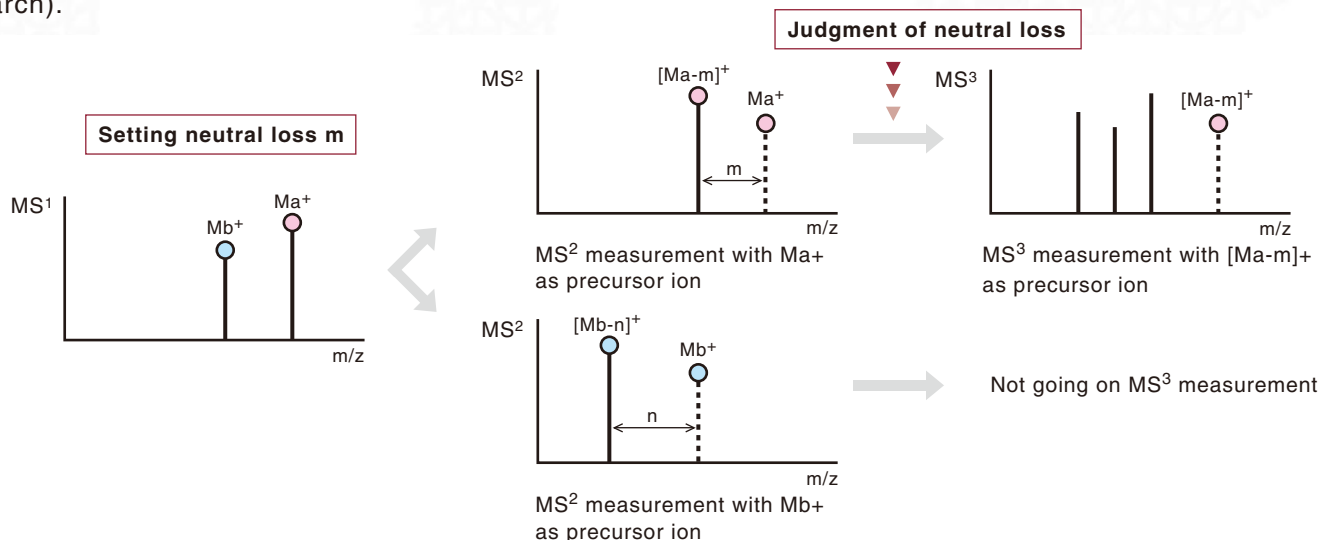
TIC

MS^1

Threshold

m/z

MS^1

Threshold

m/z

Selecting precursor ions automatically and measuring MS^2

MS^2

m/z

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
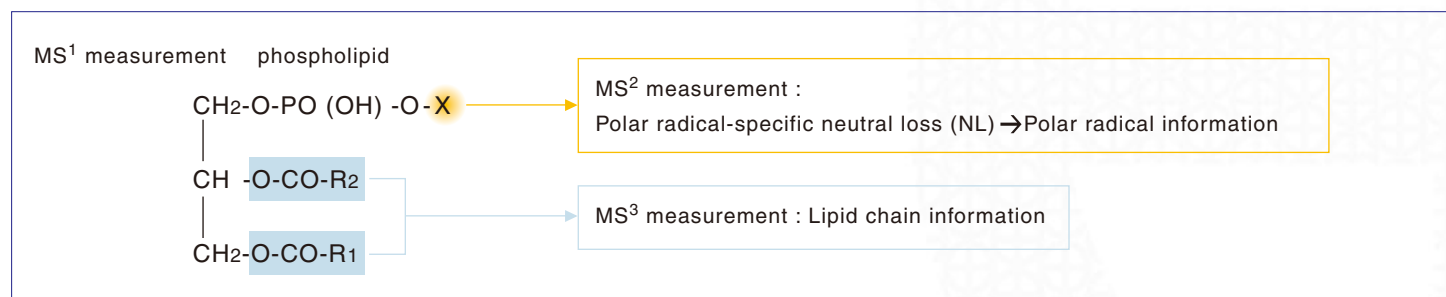- Neutral Loss Survey

Principles of Neutral Loss Survey

MS^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).
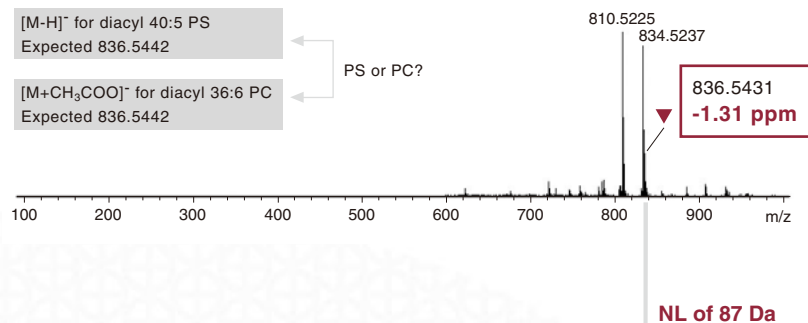
Setting neutral loss m

MS^1

Mb$^+$

Ma$^+$

m/z

Judgment of neutral loss

MS^2

[Ma-m]$^+$

Ma$^+$

m

m/z

MS^2 measurement with Ma+ as precursor ion

MS^3

[Ma-m]$^+$

m/z

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

MS^2

[Mb-n]$^+$

Mb$^+$

n

m/z

MS^2 measurement with Mb+ as precursor ion

Not going on MS^3 measurement

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.
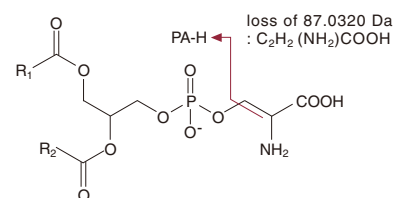
MS^1 measurement   phospholipid

CH_2-O-PO (OH) -O-**X**   →   **MS^2 measurement :**
Polar radical-specific neutral loss (NL) → Polar radical information

CH -O-CO-R$_2$

CH_2-O-CO-R$_1$ →   MS^3 measurement : Lipid chain information

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

MS^1

[M-H]$^-$ for diacyl 40:5 PS
Expected 836.5442

[M+CH$_3$COO]$^-$ for diacyl 36:6 PC
Expected 836.5442

PS or PC?

810.5225
834.5237

836.5431
-1.31 ppm

NL of 87 Da

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

loss of 87.0320 Da
: C$_2$H$_2$ (NH$_2$)COOH

PA-H

COOH

NH$_2$
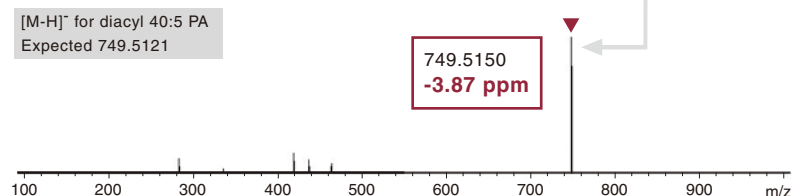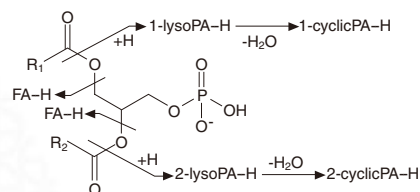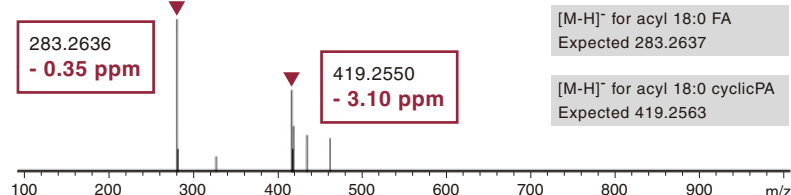
Detection of PS-specific NL: Polar radical information

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

MS^2 of m/z 836.5

[M-H]$^-$ for diacyl 40:5 PA
Expected 749.5121

749.5150
-3.87 ppm

1-lysoPA–H → 1-cyclicPA–H
-H$_2$O

FA–H
FA–H

2-lysoPA–H → 2-cyclicPA–H
-H$_2$O

MS^3 of m/z 749.5

283.2636
- 0.35 ppm

419.2550
- 3.10 ppm

[M-H]$^-$ for acyl 18:0 FA
Expected 283.2637

[M-H]$^-$ for acyl 18:0 cyclicPA
Expected 419.2563

MS^3 measurement: Lipid chain information

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.

Sample   : lipid mixture from rat liver
Column   : Si column (1.0 x 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)

Highly Reliable Protein Analysis

## Protein Analysis Software (Optional product)

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

Protein Analysis 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 Analysis 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.
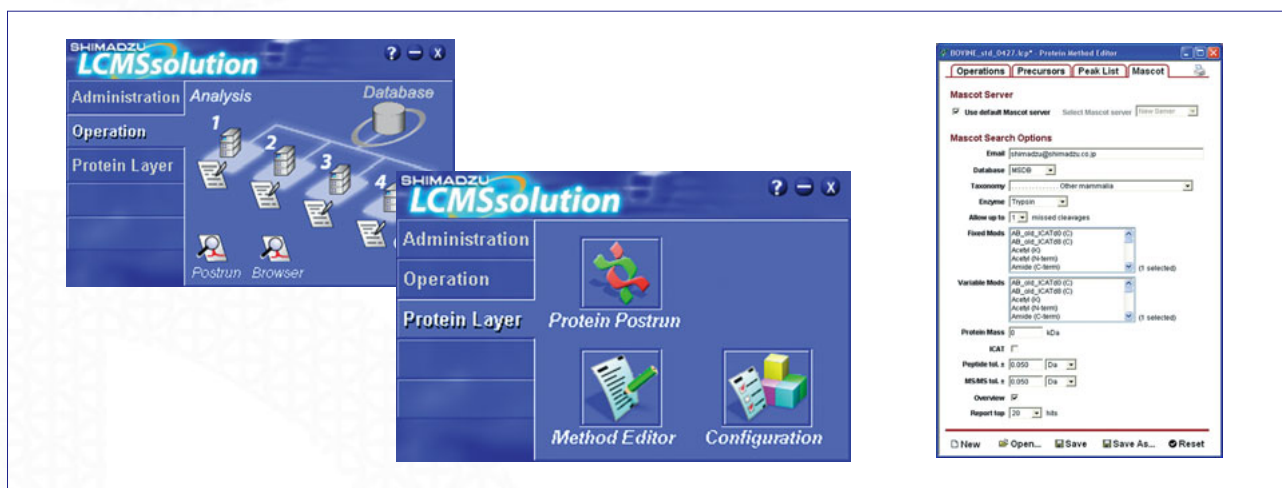
NES-100 NanoESI Interface (Optional product)

Higher sensitivity with higher accuracy of LCMS-IT-TOF® data

The NES-100 is installed in the LCMS-IT-TOF to enable analysis with nanoHPLC.
Nano-spray increases ionization efficiency by reducing loss in ionization.
Even ultra-trace samples can be analyzed with greater certainty and higher sensitivity using the NanoESI interface.
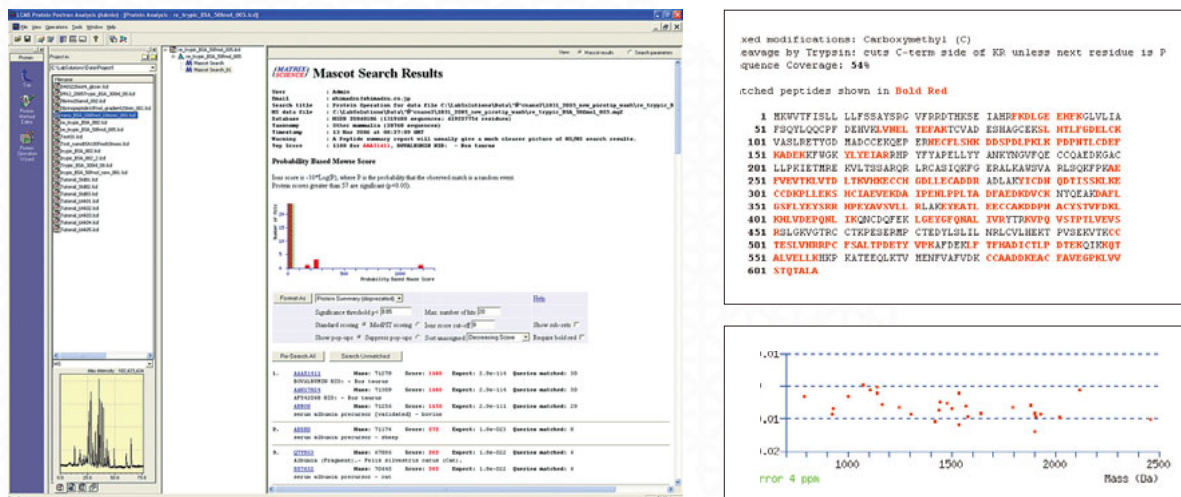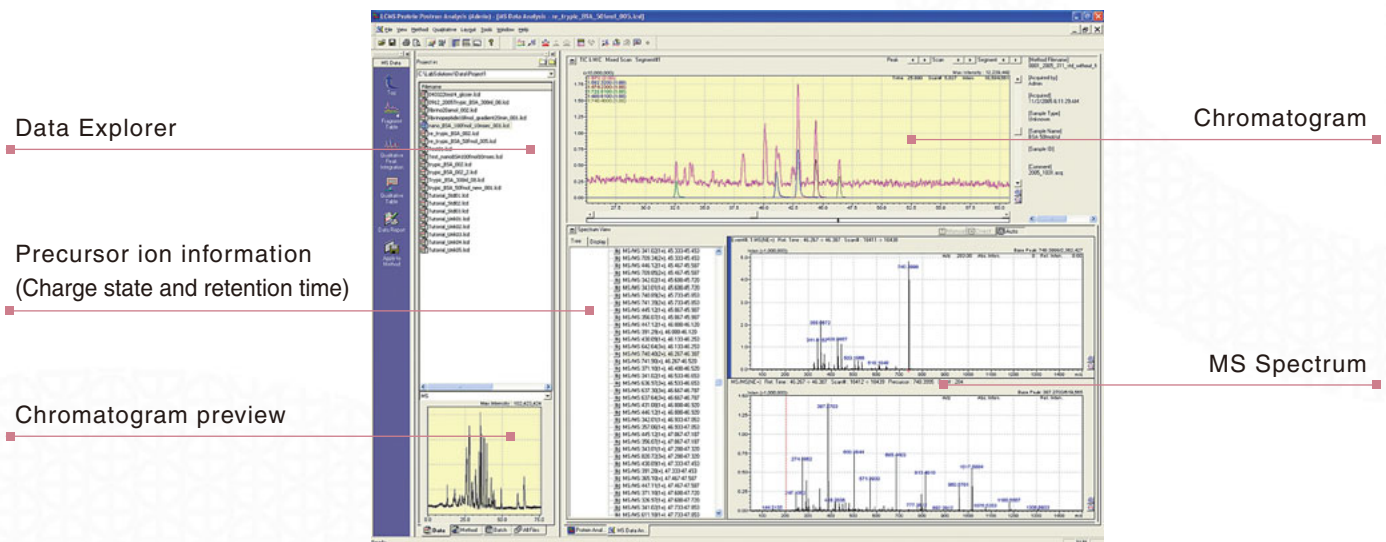This easy-to-use interface can be attached the same way as the standard ESI interface.

Measurement Example (BSA Tryptic digest 50 fmol)

When a protein enzyme digest is separated using HPLC, it is common to see tens to hundreds of peaks corresponding to various peptides. Additionally, it is not rare for several peptides to be eluted within a period of just a few seconds.

In this situation, the sequence coverage can be improved using high-speed and intelligent auto MS^n analysis. As seen from the high-scoring MASCOT hits, the quality of MS spectra achievable with the LCMS-IT-TOF® can improve your protein analysis reliability.

A screen capture of the Protein Analysis software

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

Data Explorer

Precursor ion information
(Charge state and retention time)

Chromatogram preview

Chromatogram

MS Spectrum

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).

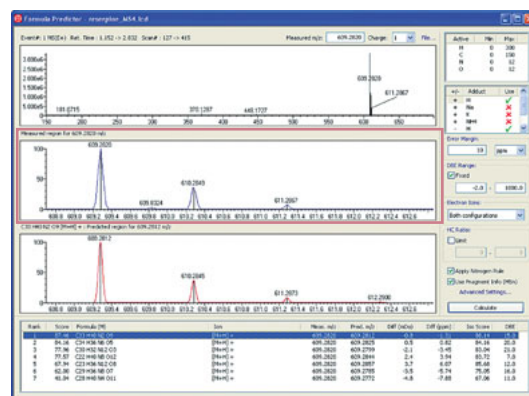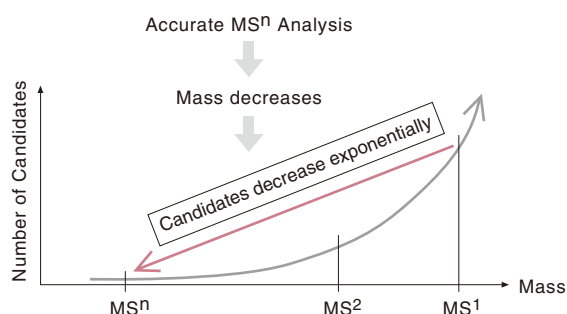This data exemplifies the high-quality measurement obtainable with the high-accuracy LCMS-IT-TOF®.

Software to Accelerate Data Analysis

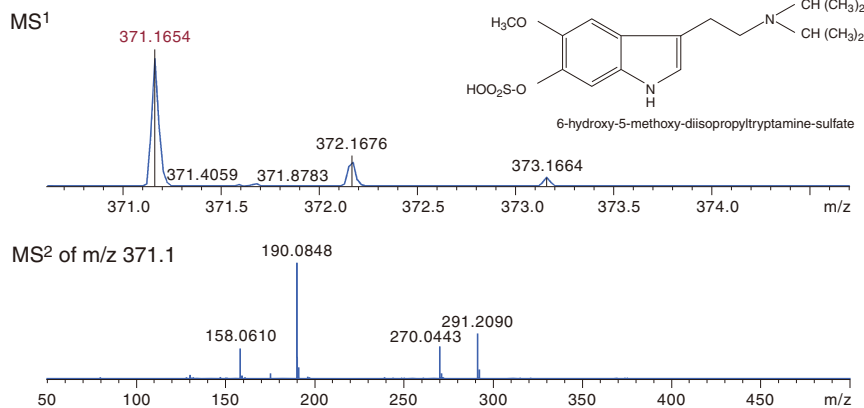## Composition Prediction Software (Optional product)

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 the Composition Prediction Software, 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.

Accurate MS^n Analysis

Mass decreases

Candidates decrease exponentially

Number of Candidates

MS^n MS^2 MS^1 Mass

C$_{17}$H$_{26}$N$_2$O$_5$S+H$^+$ (m/z 371.1641) Composition Prediction Example

MS^1

371.1654

372.1676

371.4059   371.8783

373.1664

371.0   371.5   372.0   372.5   373.0   373.5   374.0   m/z

H$_3$CO

HOO$_2$S-O

CH (CH$_3$)$_2$
CH (CH$_3$)$_2$

6-hydroxy-5-methoxy-diisopropyltryptamine-sulfate

MS^2 of m/z 371.1

190.0848

158.0610

291.2090

270.0443

50   100   150   200   250   300   350   400   450   m/z

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$_2$O$_5$S+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.

(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		

S

MetID Solution Metabolite Structural Analysis Software (Optional product)

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.
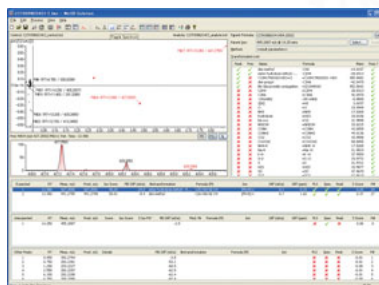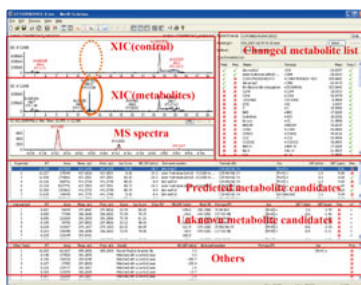
Profiler™ AM$^+$ Metabolomics Analysis Software (Optional product)

Fusion of Accurate MS^n Capability and Informatics

The fusion of the LCMS-IT-TOF®, with its high speed and mass accuracy, together with a multivariate statistical analysis program called Profiler-M, which is provided by Phenomenome Discoveries Inc. (PDI), enables fast extraction of the changes between compounds from data sets of complex biological samples, followed by linking them with identifications.
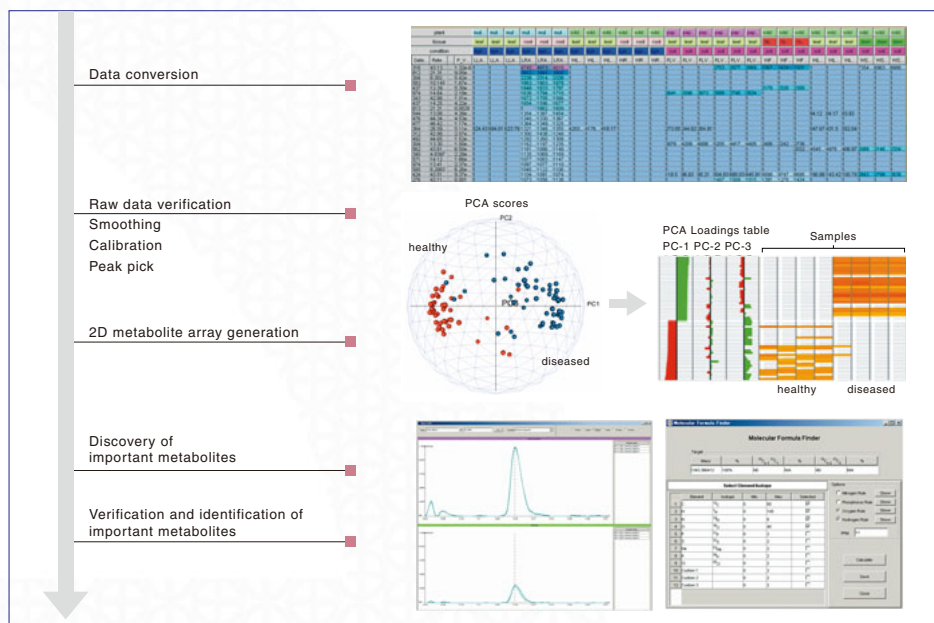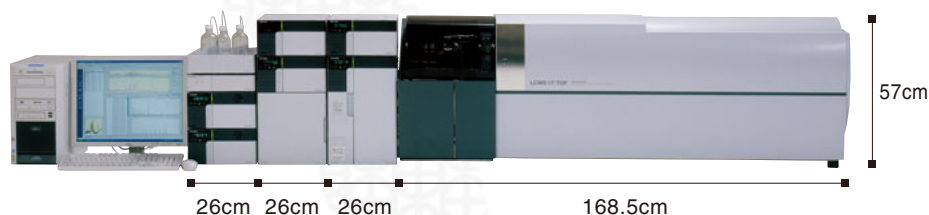
Prominence Series
Semi-micron high-pressure
liquid chromatography front end system

+ LCMS-IT-TOF ®

Installation View

57cm

26cm 26cm 26cm   168.5cm

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

ISO 9001

JQA-0376

⚠Warning		⚠Warning
Shock hazard		High-temperature hazard
Danger of electric shock.Turn power OFF prior to opening cover.		Danger of burns. Do not touch or look through.

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