Applications note

Ultra-High Mass-Resolution
MALDI-TOFMS System

Solutions for Innovation

JMS-S3000 SpiralTOF™ series

Shared Instrument Applications Notebook

Edition August 2023

JEOL Ltd.

JMS-S3000 SpiralTOF™ series

Shared Instrument Applications Notebook

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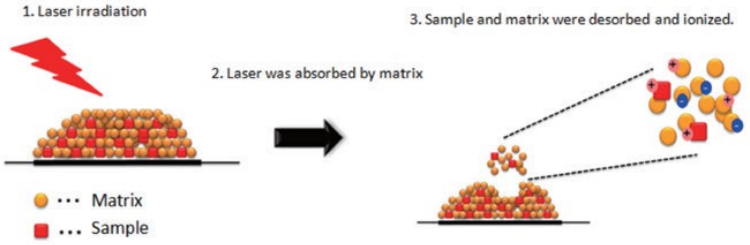
JMS-S3000 SpiralTOF™-plus 2.0 MALDI-TOFMS as a Shared Instrument

What is MALDI?

MALDI is a typical soft ionization method for mass spectrometry. A solution of ionization promoter called matrix and sample solution are mixed and dropped onto a stainless 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 – from a few hundred Daltons to hundreds of thousands of Daltons. Therefore, it is most commonly combined with a time-of-flight mass spectrometer (TOFMS), which has an inherently wide mass (*m/z*) range. Because singly charged ions are primarily produced, the horizontal axis equals the mass of molecule, making it easy to interpret mass spectral data.



Application range of MALDI-TOFMS

Due to the history of its invention, MALDI tends to be emphasized as an analysis method for macromolecules such as proteins and synthetic polymers. In fact, it is a versatile ionization method that can ionize a wide range of molecules, from small molecules of < 100 Da to macromolecules of > 100,000 Da, as long as the analyte is nonvolatile. However, in combination with the conventional reflectron TOFMS, there were the following issues regarding the analysis of small to medium molecules.

- Due to post-source decay (PSD) of matrix-derived ions, there is a lot of chemical noise in the low *m/z* region, which interferes with ions derived from small molecules.
- It is difficult to obtain sufficient mass measurement accuracy to determine the elemental composition required for the analysis of small to medium molecules.

The JMS-S3000 SpiralTOF™ series has solved the above problems by adopting JEOL's unique SpiralTOF ion optics and fully realized the versatility inherent in MALDI. JMS-S3000 SpiralTOF™-plus 2.0 is ideal as a shared instrument since:

- MALDI is a direct analysis method without chromatographic separation – molecular weight information can be obtained quickly.
- Carryover and interference between users can be minimized by properly managing target plates

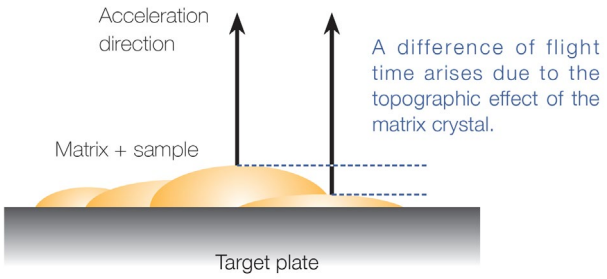
JMS-S3000 SpiralTOF™ series spiral ion trajectory time-of-flight mass spectrometer

To improve the mass resolving power and mass accuracy of a time-of-flight mass spectrometer, the flight distance must be extended while keeping a group of ions having the same *m/z* (an ion packet) from diverging in space. For the SpiralTOF™ series, the innovative SpiralTOF ion optics was developed by JEOL[1] based on the “Perfect focusing[2]” and “Multi-turn[3]” principles and a spiral ion trajectory of 17 m was realized in a limited space. The SpiralTOF ion optics is the optimum ion optics for MALDI-TOFMS, which can simultaneously achieve high mass-resolution and high mass-accuracy over a wide mass range, which was difficult with conventional reflectron TOFMS. Since ions derived from post source decay, which is unique to MALDI, can be eliminated by sector electric fields, mass spectra with good S/N can be obtained even in the low-molecular-weight region.

[1] Japanese patents JP4980583, JP5238054, JP5226824 U.S. patents US7504620, US7910879, US8237112 (as of July 2023)
[2] Perfect Spatial and Isochronous Focusing Ion Optics for Multi-turn Time of Flight Mass Spectrometer: M. Ishihara, M. Toyoda and T. Matsuo, Int. J. Mass Spectrom., 197, 179-189, 2000
[3] Multi-turn Time-of-Flight Mass Spectrometers with Electrostatic Sectors: M. Toyoda, D. Okumura, M. Ishihara and I. Katakuse, J. Mass Spectrom., 38, 1125-1142, 2003

Reduced topographic effect of matrix crystals

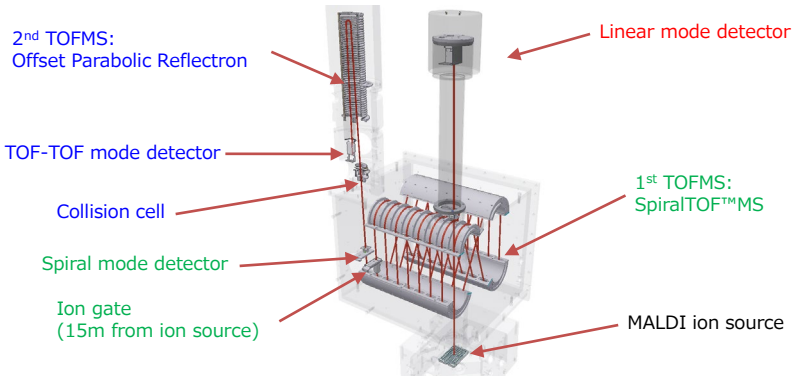
The topographic effect of the matrix crystal leads to a difference in flight start position for the ions, resulting in lower mass-resolving power. With its extended flight distance, the SpiralTOF™ series reduces this effect to the minimum and achieves highly reproducible mass resolving power and high mass accuracy with an external mass calibration. This enables highly accurate measurements regardless of sample preparation experience, and is an important performance feature for a shared instrument.



Three modes that characterize the JMS-S3000 SpiralTOF™-plus 2.0

SpiralTOF mode

Spiral trajectory ion optics have a long flight path (17 m) in a compact space. As the ions fly while being converged, high mass-resolving power, high mass-accuracy, as well as high sensitivity are achieved. In MALDI-TOFMS, the laser irradiation position is the starting point of TOFMS, so the topographic unevenness of the crystal also affects the mass spectrum quality. However, the SpiralTOF mode, which has a long flight distance, is less susceptible to this effect and does not require a know-how for high-resolution measurement. Anyone can easily perform accurate mass measurement in a wide mass range quickly, which was difficult with conventional reflectron TOFMS.



LinearTOF mode (option)

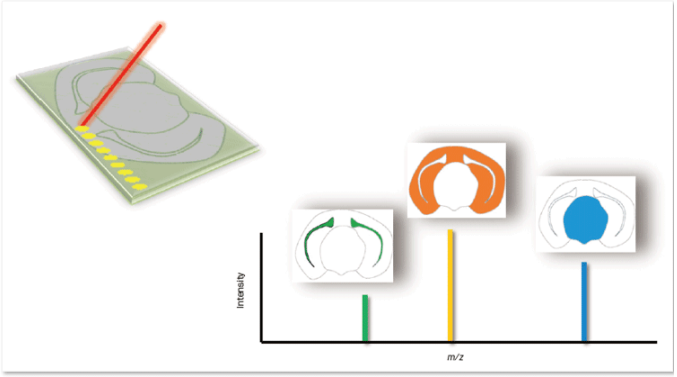
A linear ion optics. It is the simplest ion optical system and is suitable for measuring high molecular weight samples. Mainly used for the analysis of proteins and high molecular weight polymers.

TOF-TOF mode (option)

A tandem time-of-flight mass spectrometer with a SpiralTOF as the first TOFMS and a reflectron TOFMS as the second TOFMS. Structural analysis is possible from the information-rich fragmentation pattern by high-energy collision-induced dissociation.

Mass spectrometry imaging

Mass spectrometry imaging was initially developed for high-molecular-weight compounds such as proteins and peptides. However, as applications expand, analysis of low-molecular-weight compounds such as lipids, drugs, and metabolites is becoming mainstream. Conventional reflectron MALDI-TOFMS is not good at measuring low molecular weight regions that are interfered with by matrix-derived ions. In addition, mass spectrometry imaging also observes contaminants on the sample surface, increasing the number of factors that hinder observation of target compounds. Therefore, it is very important to acquire highly selective localization information with high mass resolution even in the low molecular weight region. SpiralTOF™-plus 2.0 is a MALDI-TOFMS system that makes it possible.



Analysis of medicinal properties in a combination cold remedy by using JMS-S3000 “SpiralTOF™”

Product used：Mass Spectrometer (MS)

Matrix assisted laser desorption ionization (MALDI) is a soft ionization method that uses laser energy absorbing “matrix” compounds to assist with the ionization process. Typically, the molecular weight for these matrice ions are in the low *m/z* range (typically 100-300Da range). The MALDI ion source is typically combined with reflectron type time-of-flight mass spectrometers (TOFMS), which offer insufficient mass resolving power for separating matrix and contamination peaks from analyte peaks of interest with low molecular weights. The JMS-S3000 “SpiralTOF™” (Figure 1) is a MALDI-TOFMS that has a spiral ion trajectory that folds a 17m flight path into a one meter box by using four electrostatic sectors. As a result, the SpiralTOF™ has a five times longer flight path than conventional reflectron TOFMS systems thus allowing it to achieve the highest mass resolution commercially available for a MALDI-TOFMS system. Additionally, interference from chemical background are reduced by eliminating post source decay ions in the electrostatic sectors. These features allow the SpiralTOF™ to easily detect low molecular weight ions. In this report, we will discuss the analysis of medicinal components in a combination cold remedy.

Experiments

A commercially available combination cold remedy tablet was dissolved in 13.4 ml of water. The seven medicinal ingredients in the tablet were acetaminophen, tranexamic acid, dl-methylephedrine hydrochloride, anhydrous caffeine, d-chlorpheniramine maleic acid, dihydrocodeine phosphate and isopropamide iodide. Sinapic acid ($C_{11}H_{12}O_5$) was used for matrix. The SpiralTOF™ was used in positive ion mode to acquire the sample mass spectra.

Results

The mass spectrum of the combination cold remedy is shown in Figure 2. All of the ions related to the seven medicinal components were observed as $[M+H]^+$. The mass correction for accurate mass analysis was performed by using two peaks related to the sinapic acid matrix compound $[M-H_2O+H]^+$ (*m/z* 207.0659, ▼ in Figure 2) and $[2M+Na]^+$ (*m/z* 471.1262, not shown). The observed and calculated mass for the ions related to seven medicinal components are listed in Table 1. The mass error for each measured ion was within 0.001 u of the calculated value. Additionally, high mass accuracy was achieved even for the low intensity ions such as d-chlorpheniramine and dihydrocodeine, as a result of the high mass resolution and reduction of background interferences in the low mass region.

Conclusion

In this work, we analyzed the medicinal components in a combination cold remedy tablet by using JMS-S3000 “SpiralTOF™” and showed high accuracy mass analysis in the low mass region, which is difficult to do using a conventional reflectron type TOFMS. Furthermore, we showed an easy method for getting high mass accuracy in the low mass region by using matrix peaks for internal mass correction.

a) b)

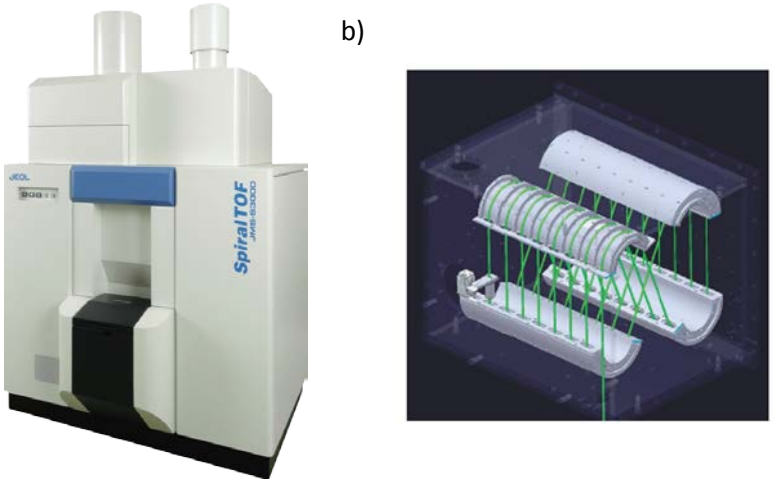


Figure 1. a) JMS-S3000 “SpiralTOF™” and b) spiral ion trajectory.

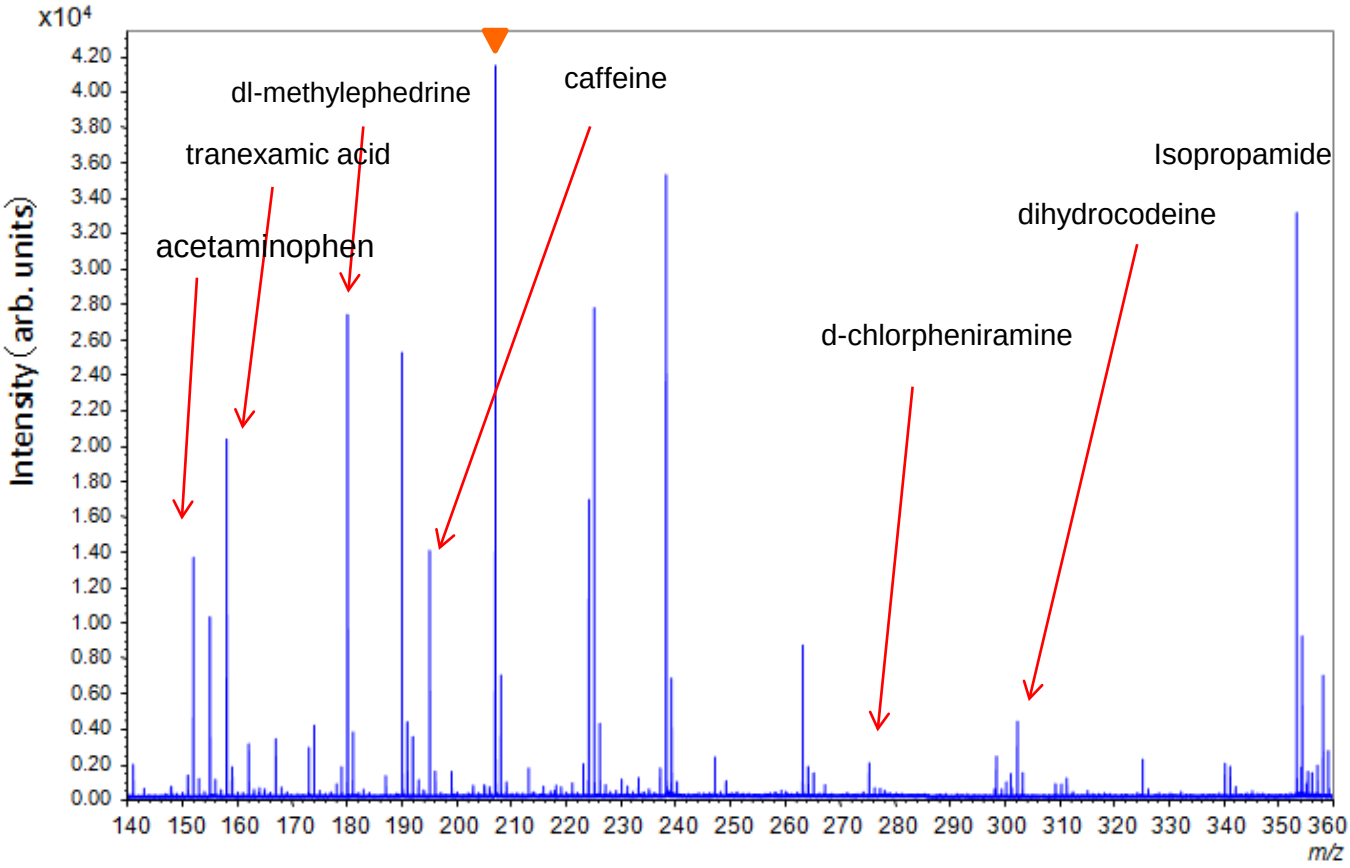


Figure 2. Mass spectrum of the combination cold remedy

Table1. Mass accuracy of the medicinal components in the combination cold remedy.

Medicinal properties	Chemical formula	Calc.[M+H]⁺ (u)	Observed (u)	Error (u)
acetaminophen	$C_8H_9NO_2$	152.0706	152.0707	+0.0001
tranexamic acid	$C_8H_{15}NO_2$	158.1176	158.1166	-0.0010
methylephedrine	$C_{11}H_{17}NO$	180.1383	180.1375	-0.0008
caffeine	$C_8H_{10}N_4O_2$	195.0877	195.0880	+0.0003
hlorpheniramine	$C_{16}H_{19}ClN_2$	275.1310	275.1300	-0.0010
dihydrocodeine	$C_{18}H_{23}NO_3$	302.1751	302.1747	-0.0004
Isopropamide	$C_{23}H_{32}N_2O$	353.2587	353.2573	-0.0014

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Applications note

MS
MSTips No. 369
MALDI-TOFMS Application

Measurement of boroxine cage using JMS-S3000 “SpiralTOF™”

Product used : Mass spectrometry (MS)

Boroxine cages are nanometer-sized covalent cage-like molecules that utilize boroxine formation reactions [1]. Such molecular-sized hollow structures can contain other molecules. Encapsulated molecules sometimes change their properties significantly, and various applications using them are being investigated. One of the methods for confirming the synthesis of boroxine cages is mass spectrometry, and MALDI-TOFMS is suitable because it can ionize a wide range of compounds mainly as single-charge ions. The JMS-S3000 SpiralTOF™ achieves a long flight distance of 17 m due to its unique spiral ion trajectory, and can measure low-molecular-weight to high-molecular-weight compounds ionized by the MALDI method with high mass resolution and high mass accuracy. In this report, we report the accurate mass measurement of the boroxine caged 12-mer.

Measurement conditions

The sample is a boroxine caged 12-mer (Figure 1). Table 1 shows the elemental composition and mass information. DCTB (trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile) was used as the matrix, and AgTFA (silver trifluoroacetate) was used as the cationizing agent. Mass spectra were acquired in SpiralTOF mode (positive ion mode). For accurate mass measurement, mass calibration was performed using polystyrene as an internal standard.

Table1 Elemental composition and *m/z* of boroxine cage 12-mer.

Elemental composition	Lowest *m/z* of $[M+Ag]^+$	Most abundant *m/z* of $[M+Ag]^+$
$C_{600}H_{912}B_{24}O_{48}$	9246.07275 u	9261.05893 u

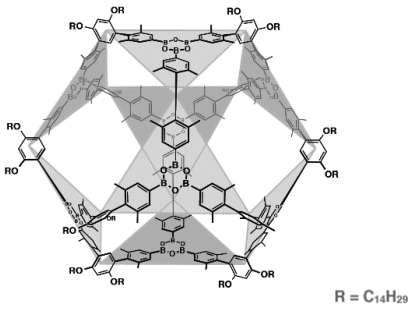


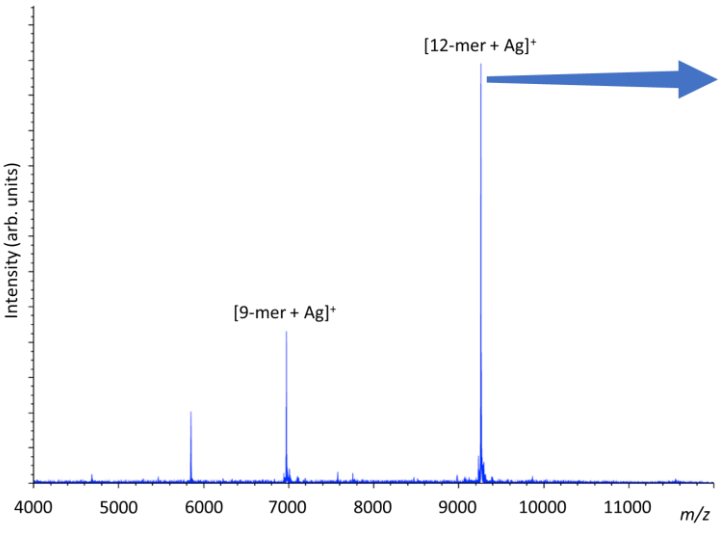
Figure1 Structure of boroxine cage 12-mer.

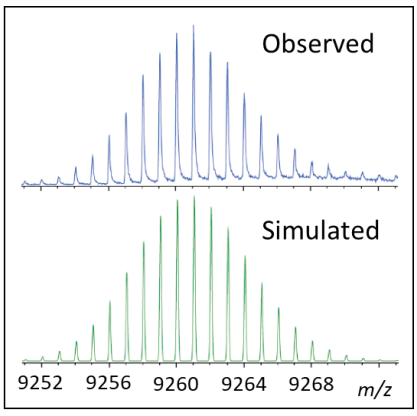
Measurement results and summary

Figure 2 shows the mass spectrum in SpiralTOF mode. In the mass spectrum, the boroxine cage 12-mer $[M+Ag]^+$ was observed as the base peak, and the 9-mer $[M+Ag]^+$ was also observed. A close-up view around the isotope peaks of the 12-mer is shown. As can be seen from this, the relative intensity of the monoisotopic peak (*m/z* 9246) is only 0.0017% in the isotope pattern of the 12-mer with a molecular weight of about 10,000 and cannot be observed. Similarly, the $[M+Ag]^+$ ion of polystyrene used as an internal standard has a low monoisotopic peak intensity, making accurate mass calibration difficult.

Therefore, isotopic peak patterns were simulated for each isotopic cluster of polystyrene used as an internal standard, and the most abundant peak within each isotopic cluster was used to establish a mass calibration. Based on this, the mass error of the most abundant peak of the boroxine-cage 12-mer isotopic cluster was examined, and the error was -0.7 mDa from the calculated mass. It also showed good agreement compared with the simulation results of isotope patterns.

In this way, SpiralTOF™ is an effective means of confirming the synthesis of high-molecular-weight compounds with a molecular weight of ~10,000 Da based on the accurate mass and isotope pattern.





Most abundant ion	
Calculated *m/z*	9261.05893 u
Observed *m/z*	9261.05966 u
Error (*m/z*)	-0.00073 u

Figure2 Observed and simulated mass spectrum of the boroxine cage 12-mer.

Reference

1) Self-Assembly of Nanometer-Sized Boroxine Cages from Diboronic Acids, K. Ono *et. al.* J. Am. Chem. Soc. 2015, 137, 7015−7018

Acknowledgement

Sample courtesy of Prof. Iwasawa, The Tokyo Institute of Technology

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Applications note MS
MSTips No. 365
MALDI-TOFMS Application

Structural analysis of a small molecule using JMS-S3000 “SpiralTOF™-plus 2.0” — MS/MS measurement of a photodegradation product of reserpine —

Instrument: Mass Spectrometer (MS)

High mass-resolution MALDI-TOFMS can determine the elemental compositions of target compounds from their measured accurate masses. MS/MS measurement is effective in elucidating the chemical structure, which is difficult to infer from accurate mass information. MS/MS measurement using JMS-S3000 “SpiralTOF™-plus 2.0” with a TOF-TOF option can detect fragment ions generated by high-energy collision-induced dissociation(HE-CID). HE-CID is a technique of fragmenting precursor ions by a single collision. It is known to provide more structural information than low-energy collision-induced dissociation, which fragments precursor ions by multiple collisions. On the other hand, since MALDI-TOFMS is difficult to connect online with pre-separation methods such as liquid chromatography, the target compounds must be separated by only their masses. High precursor ion selectivity is required to obtain accurate structural information for components that are close in their masses. SpiralTOF™-plus 2.0 with TOF-TOF option can achieve high precursor ion selectivity due to the long flight path of the spiral ion optics used in the 1st TOFMS. In this application note, we report the structural elucidation of a photodegradation product of reserpine as an example of structural analysis of a component close to a possible interference in mass by MS/MS measurement using the SpiralTOF™-plus 2.0 with TOF-TOF option.

Measurement conditions

A reserpine solution was exposed to sunlight in a room for a few days. Sample preparation method and measurement conditions were shown in Table 1. The mixture of sample and matrix solutions were spotted on a target plate and air-dried. The MS and MS/MS measurements were performed on the same sample spot.

Table 1. Sample preparation and measurement conditions

Sample	Reserpine, 1mg/THF solution
Degradation method	Daylight (A few days, Indoor)
Matrix	DHB, 10mg/mL THF solution
Spotting method	Sample + Matrix 1:10 mixed solution was spotted on the target plate
Mass spectrometer	JMS-S3000 SpiralTOF™-plus 2.0
Measurement mode	Exact mass measurement: Spiral mode, MS/MS measurement : TOF-TOF mode
Mass calibrant	PPG 700, 10mg/mL THF solution

Results

Figure 1 shows measurement results in spiral mode. Protonated molecules $[M+H]^+$ of reserpine and its photodegradation product were detected at *m/z* 609 and *m/z* 607, which are 2 u apart, respectively. MS/MS measurements were performed for *m/z* 607 and *m/z* 609, and product ion mass spectra shown in figures 2a and 3a were obtained. Na^+ or K^+ were not present in both product ion mass spectra, confirming that the precursor ions were $[M+H]^+$. Applying the adduct ion information to elemental composition estimation conditions, *m/z* 609 and 607 were matched to reserpine and dehydrogenated reserpine, respectively (Figures 2b and 3b). Furthermore, it was surmised that the double bond position in the photodegradation product of reserpine was located in the heterocyclic portion (Figures 2c and 3c).

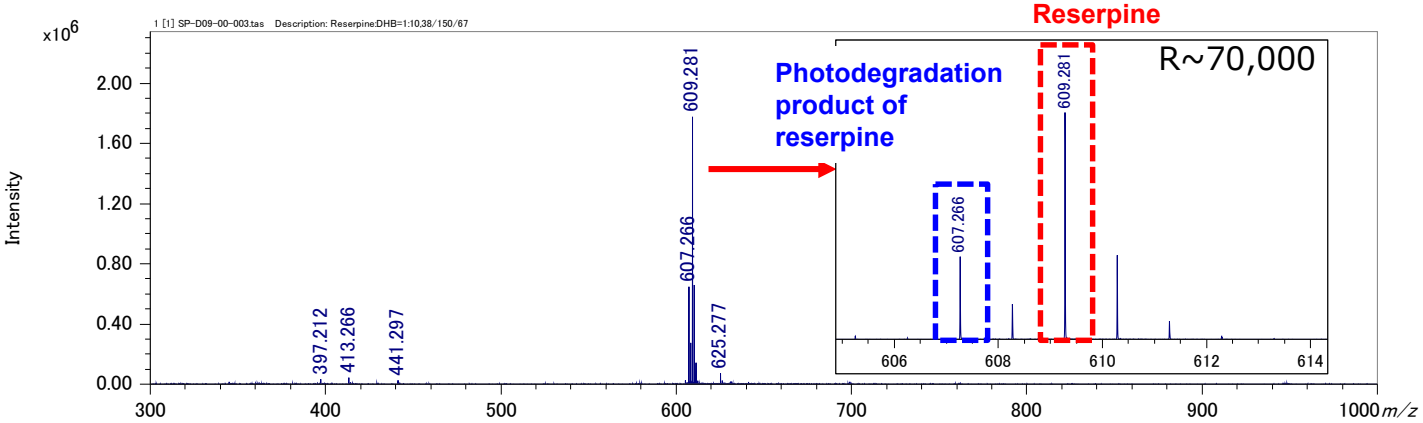
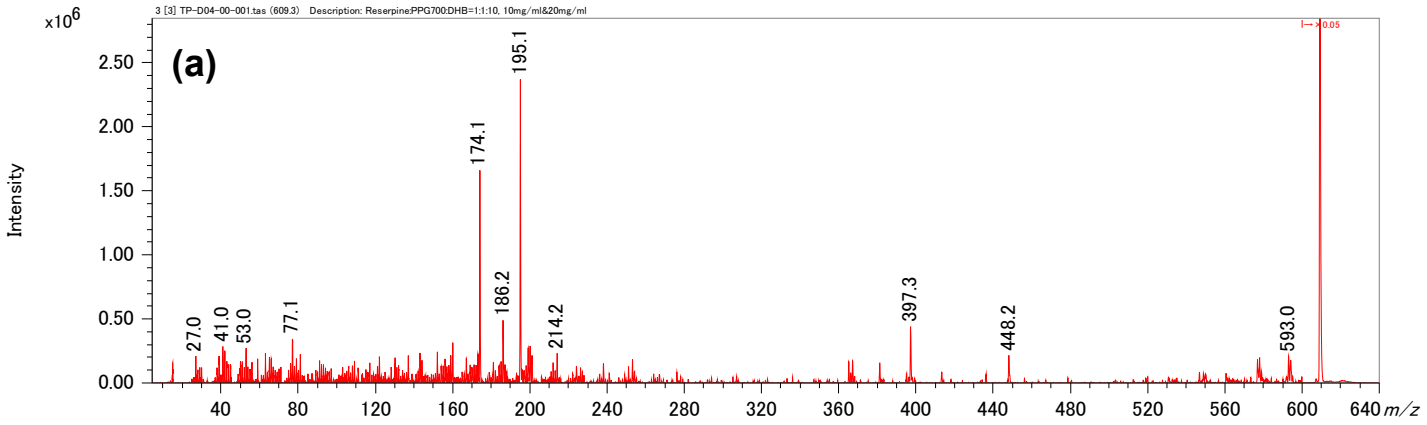


Figure 1. Mass spectrum of reserpine and its photo-degraded compound (Spiral mode)



(b)

#	Formula	Mass	DBE	Error (mDa)	Error (ppm)
1	C33 H41 N2 O9	609.2807	14.5	0.8	1.38
2	C45 H37 O2	609.2788	27.5	2.7	4.42

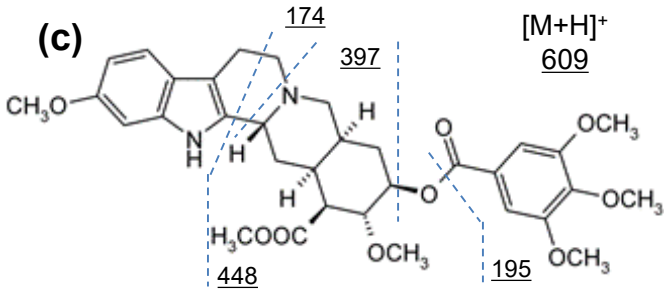
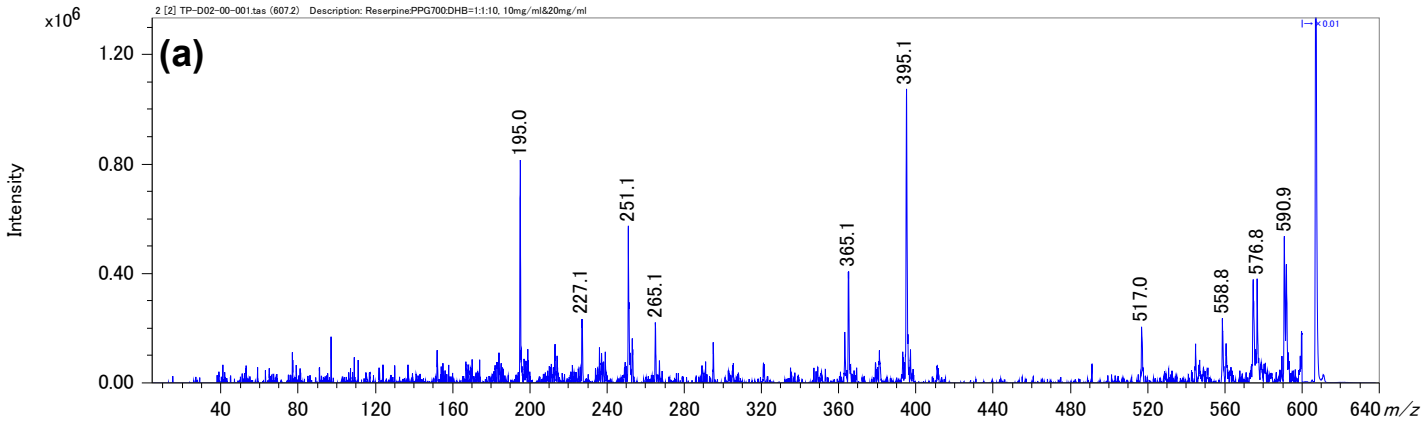


Figure 2. Product ion mass spectrum of reserpine (a). Inferred elemental composition of the ion (b) and estimated fragmentation channels(c).



(b)

#	Formula	Mass	DBE	Error (mDa)	Error (ppm)
1	C33 H39 N2 O9	607.265	15.5	0.8	1.31
2	C45 H35 O2	607.2632	28.5	2.6	4.35

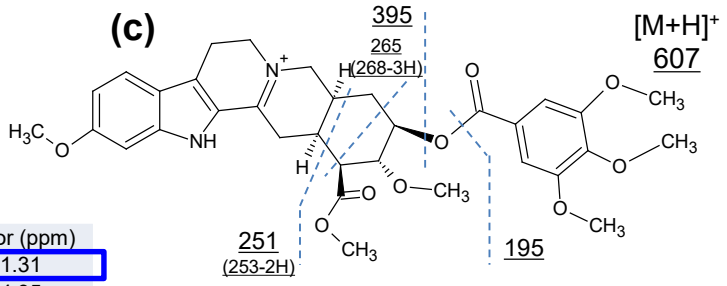


Figure 3. Product ion mass spectrum of the photodegradation product of reserpine (a). Inferred elemental composition of the ion (b) and estimated fragmentation channels(c).

Conclusion

SpiralTOF™-plus2.0 with TOF-TOF option has the high precursor ion selectivity so that the product ion mass spectra of reserpine and its photodegradation products, which differ only 2 u, were obtained separately. Adduct ions can be identified from the product ion mass spectra, and thus, it was possible to narrow down the candidates of elemental compositions. The obtained product ion mass spectra were significantly different between the two, and their chemical structures can be elucidated from unique product ion mass spectra.

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Analysis of high molecular weight polystyrene standards by using JMS-S3000 "SpiralTOF™" with Linear TOF option.

Product used：Mass Spectrometry (MS)

An example of the analysis of low molecular weight Polystyrene (PS) by using JMS-S3000 SpiralTOF™ MALDI-TOFMS was already shown in MS Tips No.163. This time, high molecular weight polystyrene standards (TSKgel Standard Polystyrene F-4 (Mw = 3.72×10^4), F-10 (Mw = 9.89×10^4), and F-20 (Mw = 1.89×10^5), Tosoh Corporation) were analyzed by using JMS-S3000 SpiralTOF™ with Linear TOF option(Fig. 1). For F-4, peaks were observed at every 104 u, which is the repeating unit of PS. For both F-10 and F-20, expected distributions around their respective average molecular weights were obtained (Fig. 2). Wide applications of the Linear TOF option for the analyses of high molecular weight synthetic polymers are expected.

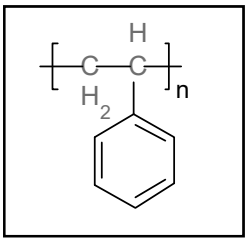

Fig.1 Repeating unit
（C_8H_8=104.0626）

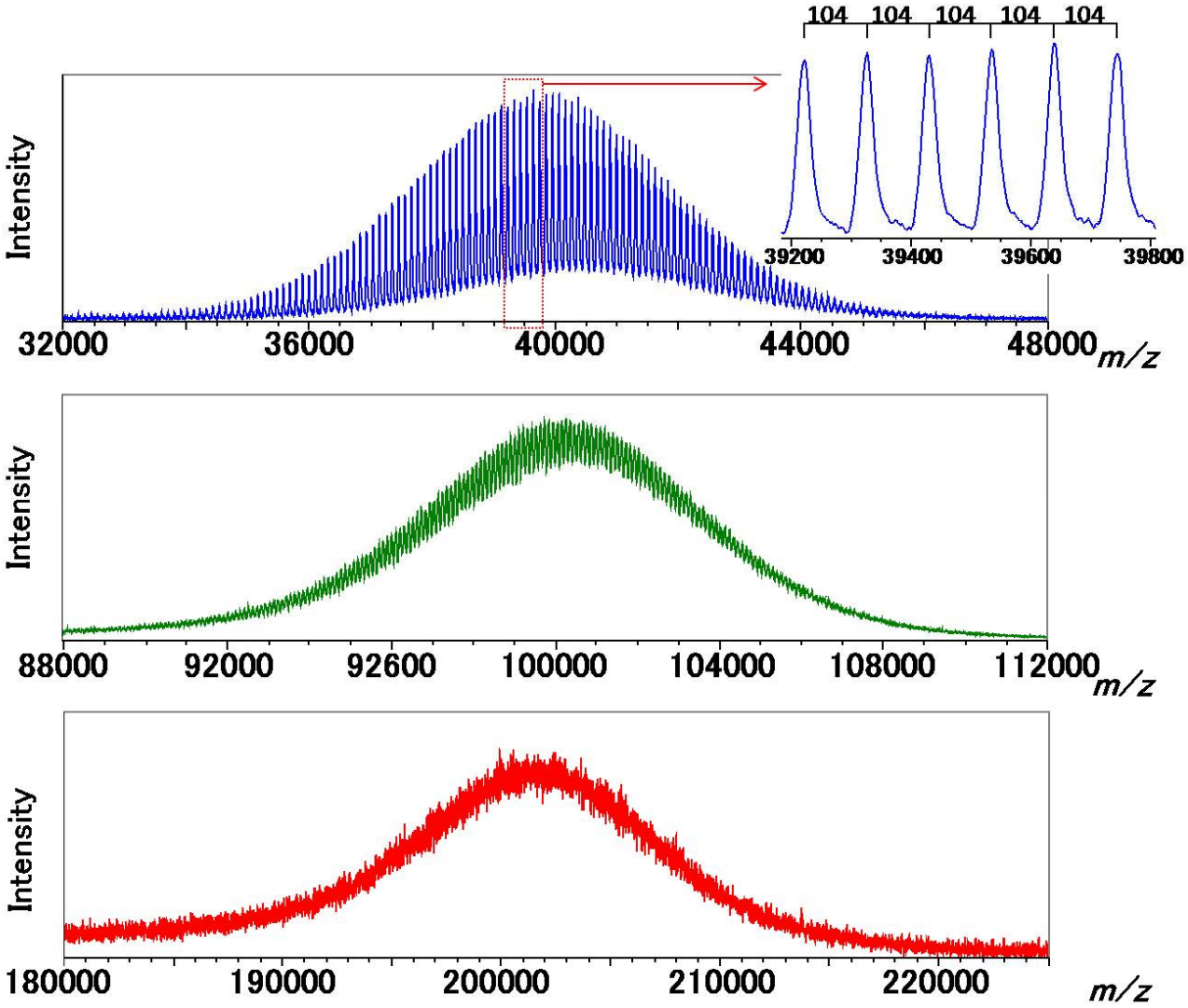

Fig.2 Mass spectra of PS using LinearTOF option.

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Applications note

MS
MSTips No. 333
MALDI Application

Structural analysis of anionic surfactants in MALDI negative ion mode using "SpiralTOF™–plus"

Product used : Mass spectrometer (MS)

The matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOFMS) is a powerful tool in analyzing polymers. Since MALDI mainly produces singly charged ions, m/z values in the mass spectrum are the same as polymer ions' mass. Using the high mass resolution MALDI-TOFMS, it is possible to identify the compositions of polymer series by their repeating unit and the end-group, and calculate the molecular weight distributions of each. The Kendrick Mass Defect (KMD) was recently applied to the polymer analysis to visualize polymer series contained in complex high-mass resolution mass spectra. In addition, the TOF-TOF option enables structural analysis, especially for end-group analysis, of polymers from fragment ions generated by high-energy collision-induced dissociation (HE-CID). In polymer analysis with MALDI-TOFMS, sample, matrix and cationizing agent solution are mixed, dropped onto a target plate, air-dried, and measured. In general, the target polymer ions will be observed in the positive ion mode as $[M+Li]^+$, $[M+Na]^+$ or $[M+K]^+$ according to the cationizing agents. However, polymers that have sulfate or phosphate end-groups can be detected in the negative ions. In this report, we will show the analysis of the mixture of anionic and nonionic surfactants.

Experiment

The sample used was a detergent containing alkyl ether sulfate (AES) and polyoxyethylene alkyl ether (POEAE) diluted 100-fold with methanol. A saturated methanol solution of α-cyano-4-hydroxysilicate (CHCA) was used for the matrix, and a 1 mg / mL tetrahydrofuran solution of sodium trifluoroacetate (NaTFA) was used as the cationizing agent. In the case of measurement in the positive ion mode, NaTFA solution was spotted first, and then a 1: 1 (v / v) mixture of the sample solution and the matrix solution was spotted and air-dried. In the negative ion mode measurement, a solution in which the sample solution and the matrix solution were mixed at a ratio of 1: 5 (v / v) was spotted and air-dried. The JMS-S3000's SpiralTOF positive/negative ion modes were used to acquire the mass spectrum, and the TOF-TOF negative ion mode was used to acquire the product ion spectrum. The msRepeatFinder was used for KMD analysis.

Result

Measurement result: Positive ion mode

Figure 1 shows (a) mass spectrum and (b) KMD plot (Base unit C2H4O) in positive ion mode. It can be seen that many components are observed in the mass spectrum of figure 1a. The separation of isobaric peaks with Δm 0.13u could be confirmed even in m/z 575 using high mass resolution MALDI-TOFMS. As a result of composition estimation from accurate mass analysis, the red and green arrows were estimated as POEAE and AES, respectively. The composition of AES was estimated as RO-(EO)n-SO3Na+Na+（R=$C_{12}H_{25}$, $C_{14}H_{29}$, EO is ethylene oxide). When the mass spectrum of the figure. 1a was visualized in KMD plot (figure 1b), two groups (colored with green and red) could be identified. The red and green groups are POEAE and AES, respectively. A polymers series containing elements with large mass defects are appeared in the upper side of the KMD plot due to the large KMD values. Therefore, AES containing sulfate (SO3Na) end-groups were plotted on the upper side of KMD plot. Using the KMD plot is advantageous, which can easily separate the polymer groups containing end-groups with significantly different elemental compositions in the complex mass spectrum.

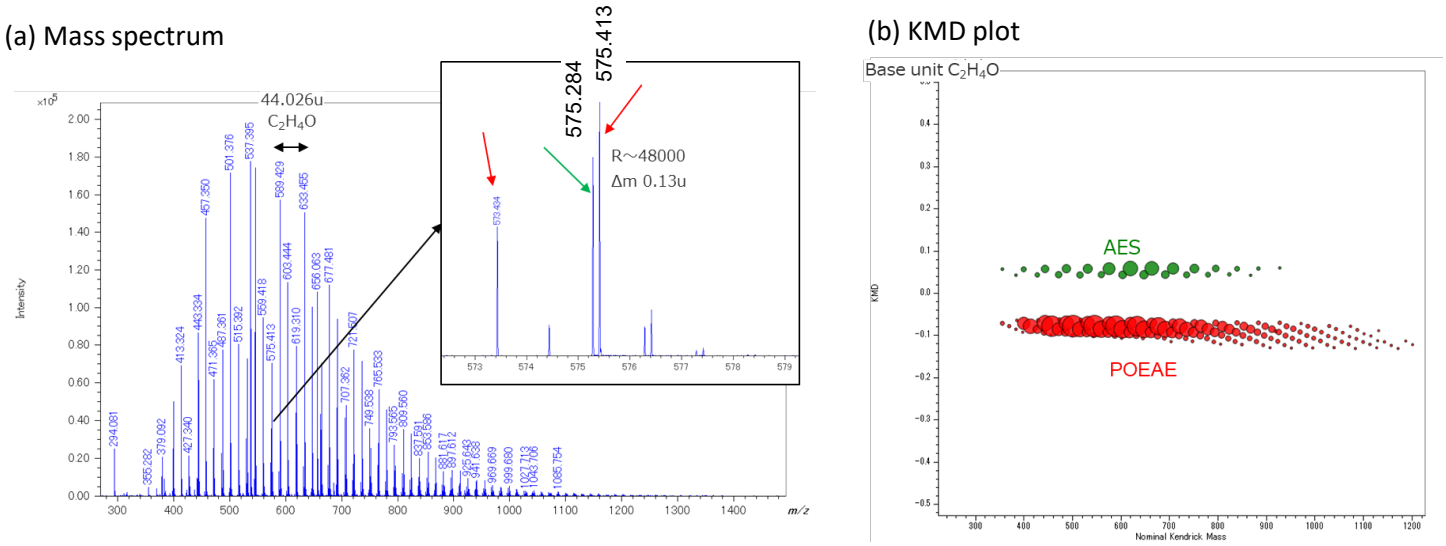

Figure 1 Positive ion mass spectrum (a) and KMD plot (b) of detergent containing AES and POEAE.

Measurement result: Negative ion mode

Figure 2 shows (a) mass spectrum and (b) KMD plot (Base unit C2H4O) in negative ion mode. In negative ion mode, only two polymer series were observed. As a result of the composition estimation, it was estimated that RO-(EO)n-SO3-（R=$C_{12}H_{25}$, $C_{14}H_{29}$, EO is ethylene oxide). It was found that AES can be selectively observed when measured in the negative ion mode. In addition, the product ion spectrum of m/z 777.5, 793.5 selected from each series were acquired by TOF-TOF mode. In the product ion spectrum, ions were observed at m/z 80, 97, which correspond to SO_3- and SO_4H-, suggesting that both series have a sulfate end-group. In addition, 198u and 170u neutral losses were observed from product ion spectra of m/z 777.5 and 793.5, respectively. They corresponded to $C_{14}H_{30}$ and $C_{12}H_{26}$, suggesting that the other end-groups are alkyl chains.

Summary

The AES was observed as RO-(EO)n-SO_3Na+Na^+ in the positive ion mode. If the sample containing AES and POEAE, the mass spectrum will be complicated because both of them can be ionized in the positive ion mode. On the other hand, AES was selectively ionized in the negative ion mode. Furthermore, end-groups were estimated by TOF-TOF negative ion mode. It was found that if an anionic surfactant such as AES is contained in the sample, it is worth trying to confirm their existence and perform structural analysis using negative ion mode.

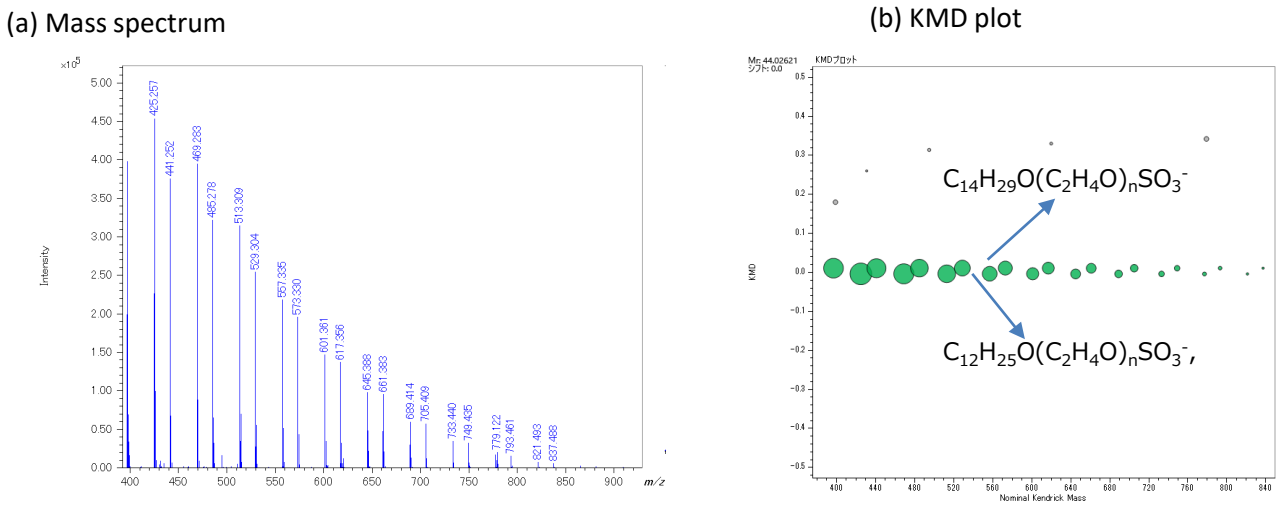

Fig. 2 Negative ion mass spectrum (a) and KMD plot (b) of detergent containing AES and POEAE.

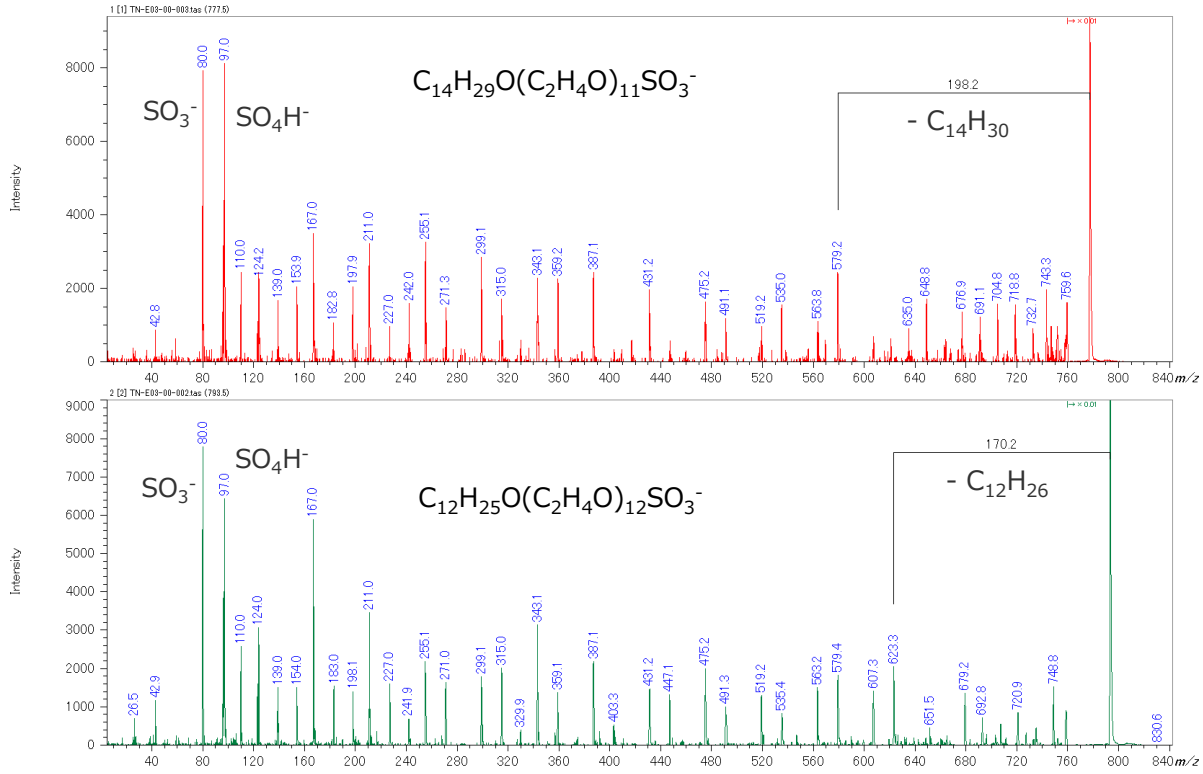

Fig. 3 Negative ion product ion mass spectra of two types of AESs.

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Applications note

MS
MSTips No. 235
MALDI Application

Analysis of low molecular weight polyethylene with solvent-free method using JMS-S3000 "SpiralTOF™"

Product used : Mass spectrometer (MS)

Matrix assisted laser desorption ionization (MALDI) time-flight mass spectrometer (TOMFS) is a powerful tool to identify the repeat units and end groups of polymers. The mass spectra of polymers can be easily interpreted because MALDI can generate singly-charged ions over a wide mass range. MALDI is a soft ionization method that uses "matrix" compounds and “cationization agents” to assist the ionization process of polymers. Typically, sample, matrix and cationization agent are dissolved in the same solvent. These solutions are pre-mixed and placed drop-wise on the target plate to make cocrystals (dried droplet method). However, this procedure cannot be applied to polymers that are insoluble or only slightly soluble. To solve this problem, solvent-free methods have been developed [1-4] for these situations. In this report, we analyzed low molecular weight polyethylene by using a solvent-free method and then using a high mass-resolution MALDI-TOFMS “SpiralTOF™” system for the analysis.

Experiments

Sample : Polyethylene analytical standard, for GPC, 1,000
(Sigma aldrich PN 81219) (PE1000)

Matrix: trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile
(DCTB)

Cationization Agent: Silver Trifluoroacetate (AgTFA)

Preparation : PE1000, DCTB、and AgTFA powders were mixed in Agate mortar. The mixed powder was then pressed onto the target plate using a spatula (Note 1). (See Figure 1)

Measurement: The mass spectra were acquired by using SpiralTOF™ positive ion mode.

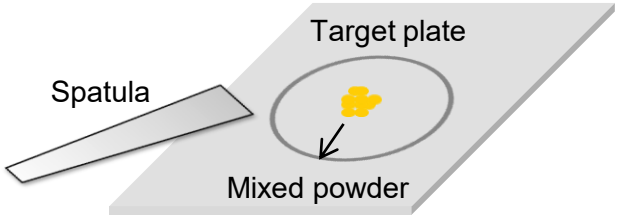

Figure 1 Solvent-free sample preparation.

Results

The PE1000 mass spectrum is shown in Figure 2. The polymer distribution of $[H(C_2H_4)_nH+Ag]^+$ was observed from 600 – 1,800 where the apex is at *m/z* 1000(Figure 2a). The enlarged mass spectrum at *m/z* 890 - 960, where $[H(C_2H_4)_nH+Ag]^+$ (n = 28 - 30) were observed, is shown in Figure 2b. The peaks had a mass resolution of more than 50,000, and the mass difference 28.031 u±0.001 u corresponded with mass of polyethylene repeat unit C_2H_4. In the solvent-free method used in this report, the mixed powder of sample, matrix, and cationization agent were pressed onto the target plate. The sample surface roughness is much larger than for the dried droplet method. Even so, the SpiralTOF™ was able to achieve high mass-resolution and mass accuracy using the system’s 17m flight path in order to overcome the adverse effects of sample roughness.

References

[1] R. Sketlton, F. Doubois, K. Zenobi, *Anal. Chem*. 72 (7) (2000) 1707–1710
[2] L. Przybilla, J. D. Brand, K. Yoshimura, H.J. Räder, K. Müllen, *Anal. Chem*. 72 (2000) 4591–4597
[3] A. Marie, F. Fournier, J.C. Tabet, *Anal. Chem*. 72 (2000)5106–5114
[4] S. Trimpin, A. Rouhanipour, R. Az, H.J. Räder, K. Müllen, *Rapid Commun. Mass Spectrom.*15 (2001) 1364–1373

(Note 1) Please press the powder firmly onto the plate in order to prevent ion source contamination from the scattering of powders.

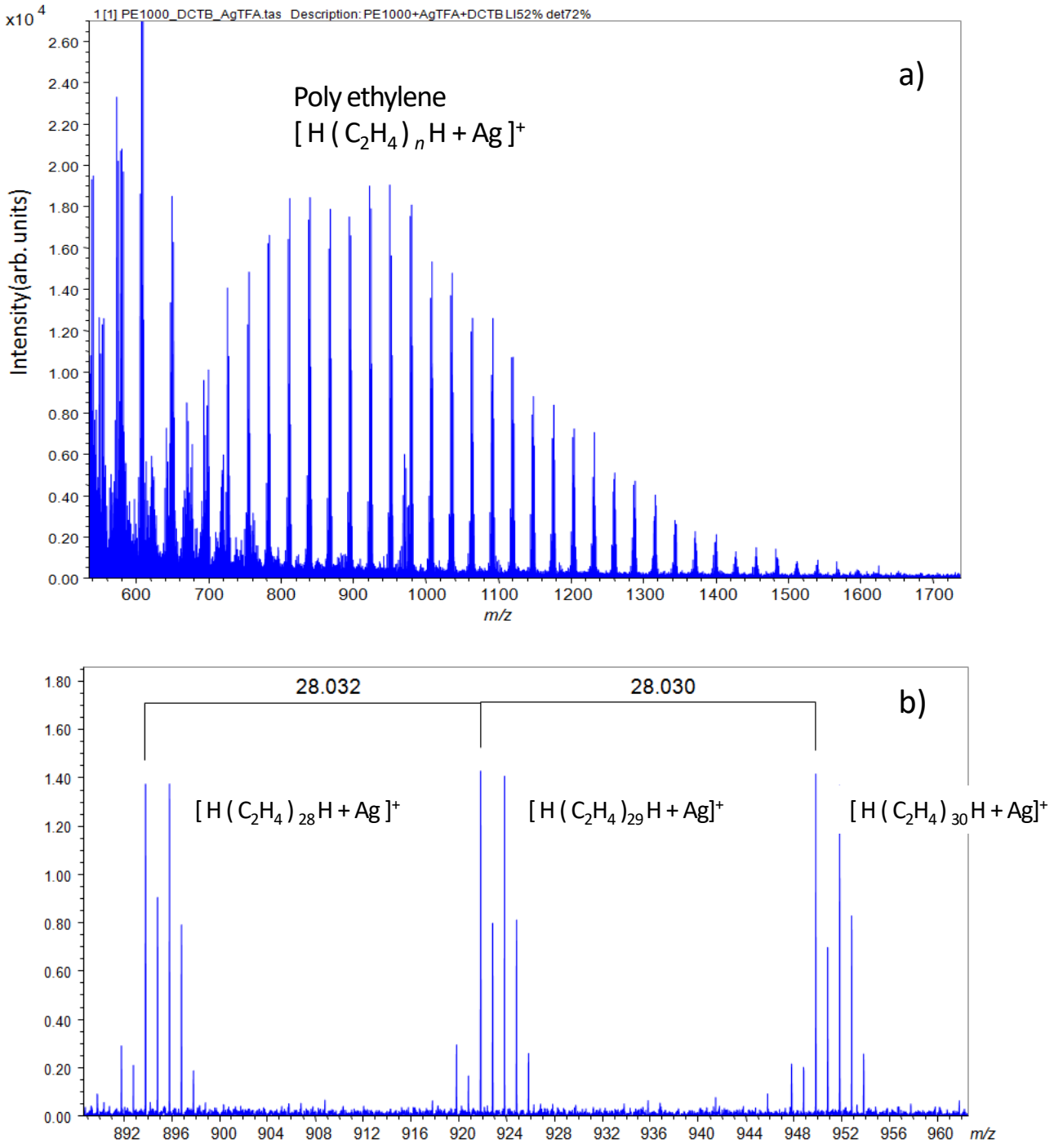

Figure 2 a) Mass spectrum of PE1000. b) Distribution of $[H (C_2H_4)_n H + Ag]^+$ was observed around *m/z* 1000.

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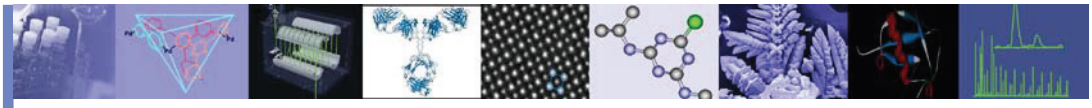


Applications Note

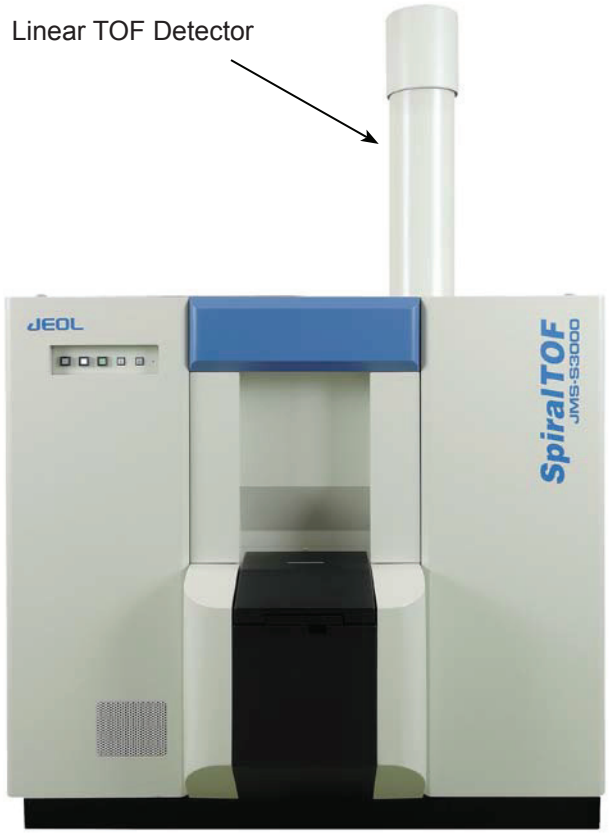
SpiralTOF™

High Sensitivity Analysis of Intact Proteins Using Linear TOF

Introduction:
The JMS-S3000 SpiralTOF has a unique 17m flight path that offers the highest resolution MALDI-TOF MS system currently available. However, ions with a very short lifetime or that undergo spontaneous dissociation during their flight cannot be detected by the SpiralTOF (or a conventional reflectron TOF). To address this situation, the SpiralTOF with Linear TOF option can be used for the high sensitivity analysis of intact proteins.

In this work, we demonstrate the measurement of intact proteins by using the Linear TOF option for the JEOL SpiralTOF system.

Experimental:
Protein standards were obtained from Sigma-Aldrich.
a. Pepsin (porcine gastric mucosa), P-6887
b. Albumin (bovine serum), A8471
c. Conalbumin (chicken egg white), C0755
d. IgG (bovine serum), I5506
The protein standard samples were dissolved in water at a concentration of 1 pmol/µL. Sinapinic acid (SA) was used as the matrix and was dissolved in 1:1 water/acetonitrile containing 0.1% trifluoroacetic acid. Next, the protein standard solution and SA solution were mixed together 1:1 by volume. Afterwards, 0.5µL of this mixture was placed on the MALDI target plate (250 fmol/spot). Finally, the dried sample was measured using the Linear TOF option available on the JMS-S3000 SpiralTOF MS system.



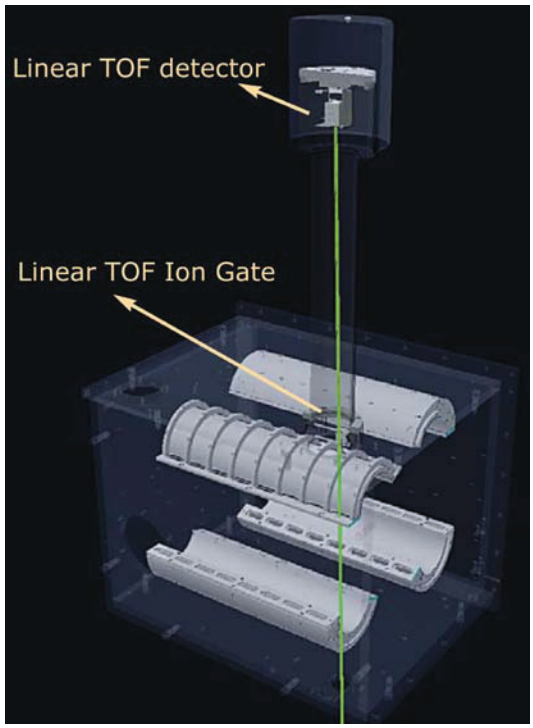


Figure 1. SpiralTOF with Linear TOF option.

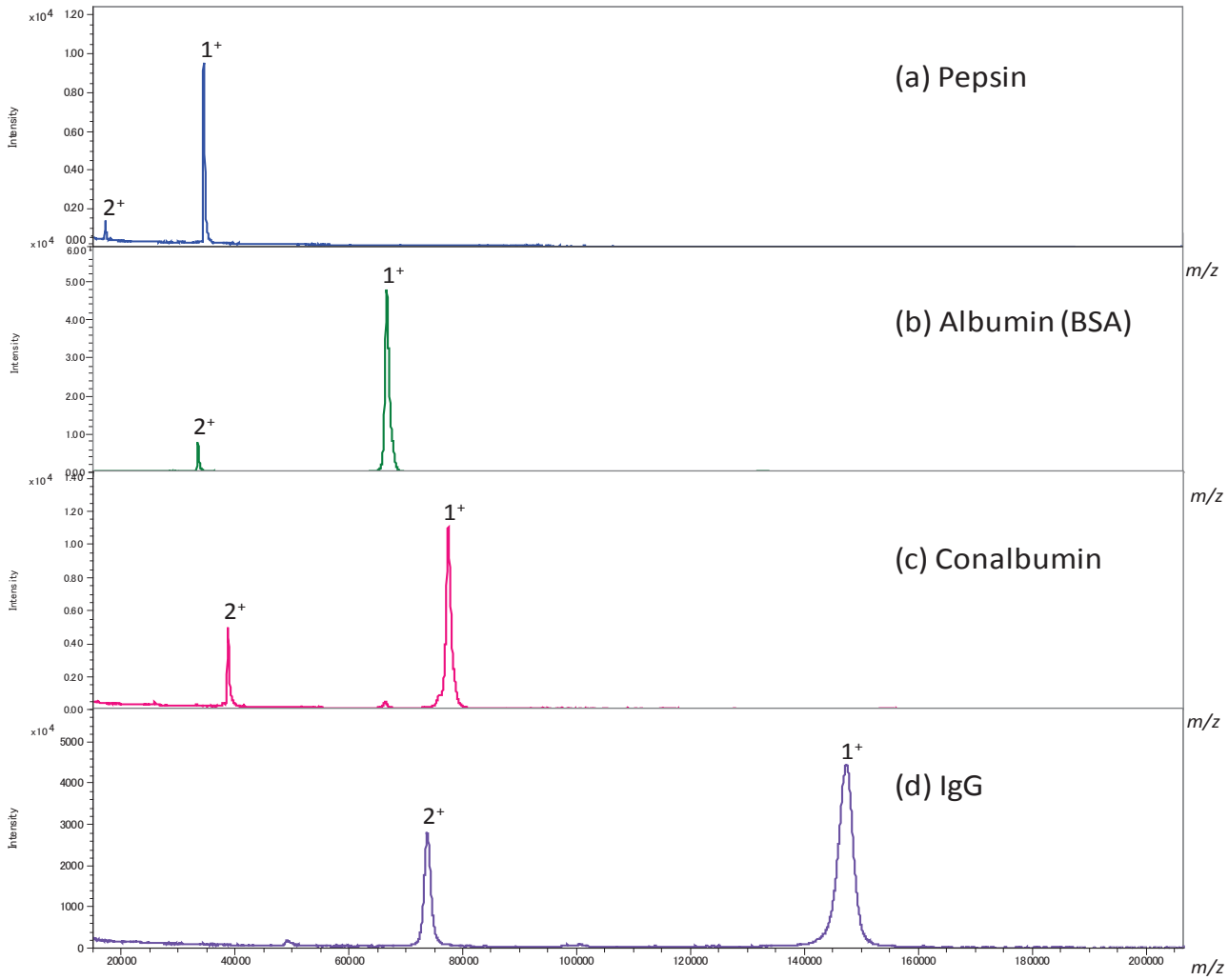
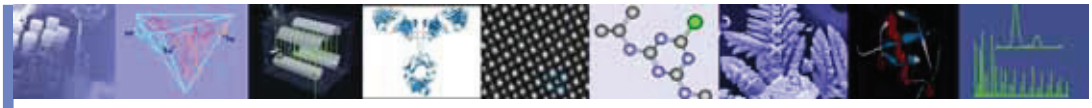


Figure 2. MALDI mass spectrum of protein standard, (a) Pepsin (250 fmol/spot), (b) Albumin (250 fmol/spot) (c) Conalbumin (250 fmol/spot), (d) IgG (250 fmol/spot).

Results & discussion:
The MALDI mass spectra are shown in Figure 2 for each protein sample. Peaks corresponding to single- and double-charge protonated molecules were observed at the expected m/z values for the primary structure of these proteins.

The SpiralTOF with Linear TOF option provided:
- Good peak shape in the high m/z region
- Excellent signal-to-noise ratio for these samples at this concentration (250 fmol)

High-quality mass spectra were achieved for intact proteins by using the JEOL JMS-S3000 system. Also worth noting, the Linear TOF can measure intact molecules up to m/z 500,000 (data not shown in these examples).



Applications Note

SpiralTOF™

Introduction of the JMS-S3000 SpiralTOF™

Analysis of Bovine Serum Albumin

Introduction:

The JMS-S3000 "SpiralTOF™" is a MALDI-TOF MS incorporating an innovative SpiralTOF™ ion optics system (Fig. 1). JEOL's patented technology[1] achieves a spiral ion trajectory of 17m within a compact 1m space. The ions are sent through four sets of layered toroidal electric fields, which are implemented by four pairs of cylindrical electrodes and nine Matsuda plates that are incorporated within every pair of cylindrical electrodes. Ions are accelerated to 20 kV in the ion source and fly sequentially through the layered toriodal electric fields to reach the detector (Fig. 2). With the extended flight distance, the JMS-S3000 SpiralTOF™ achieves high mass resolving power and high mass accuracy over a wide mass range.

However, ions with a very short lifetime or that undergo spontaneous dissociation during their flight (e.g., protonated molecules of high-mass proteins, multiply-phosphorylated peptides, etc.) cannot be detected by either the SpiralTOF™ or a conventional reflectron TOF. Considering the wide application of the MALDI technique, a mass analyzer that can detect such short-lived ions is necessary in addition to the SpiralTOF™. A Linear TOF option is available for the JMS-S3000 in order to satisfy this requirement (Fig. 2). In this application note, the analysis of the tryptic digest of bovine serum albumin (BSA) is shown as a Spiral mode example while the analysis of intact BSA is shown as a Linear mode example.

Figure 1. JMS-S3000 SpiralTOF™.

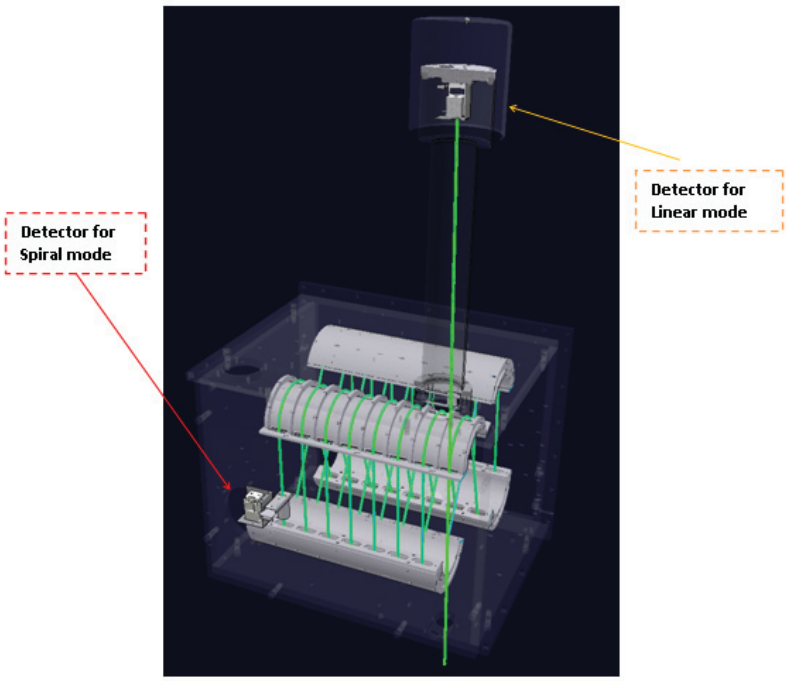


Figure 2. Ion trajectories of the JMS-S3000 SpiralTOF™.

MS-Tips #166

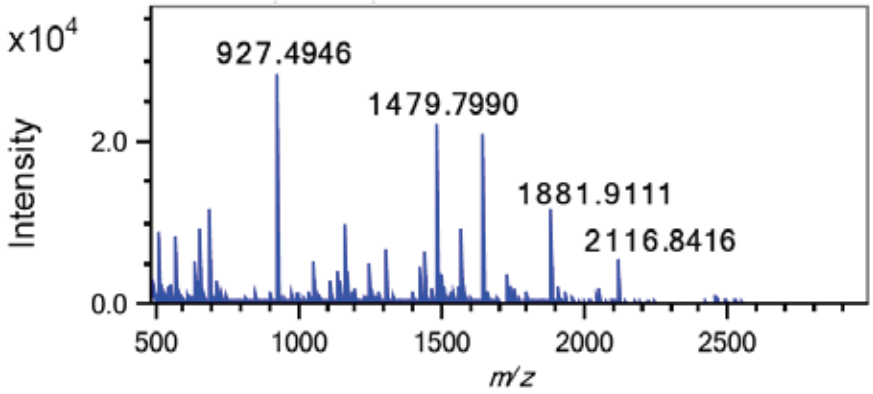


Figure 3. Mass spectrum for the tryptic digest of BSA (equivalent to 25 fmol of the protein).

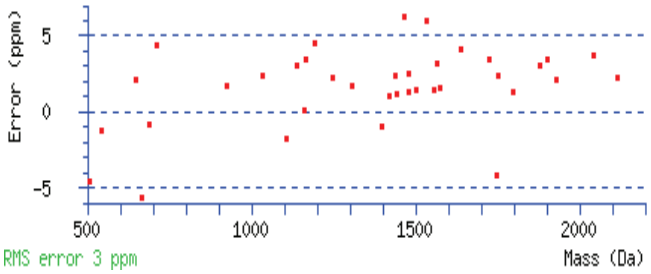


Figure 4. Mass error plot from the MASCOT PMF search result.

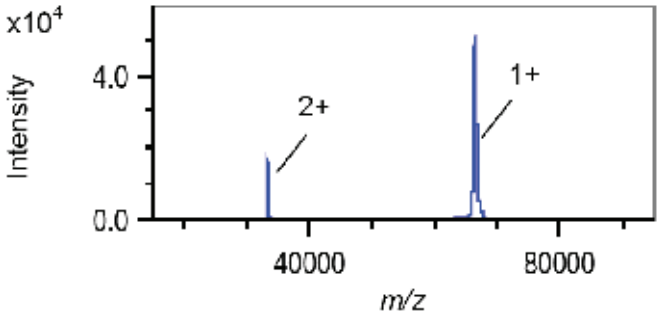


Figure 5. Mass spectrum of the intact BSA protein (2.5 pmol).

Methods:

The tryptic digest of BSA was analyzed in SpiralTOF mode. An external mass calibration was used for this data. The intact BSA was analyzed using the Linear TOF mode. The data was acquired automatically by using the msTornado™ Control, which is the instrument control and data acquisition software for the JMS-S3000.

Results and discussion:

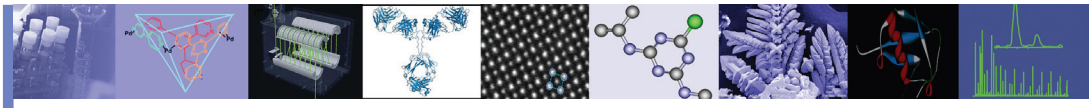
The mass spectrum of the tryptic digest equivalent to 25 fmol of BSA is shown in Fig. 3. The peak list from this mass spectrum was submitted to the MASCOT peptide mass fingerprint (PMF) search, and the protein was identified as BSA. The mass error plot of all matched peptides is shown in Fig. 4. The RMS error was 3 ppm. The mass spectrum of the intact BSA acquired in Linear mode is shown in Fig. 5. Single- and double-charge protonated molecular ion peaks were observed at the *m/z* values expected for the primary structure of this protein.

Conclusions:

A mass spectrum of the tryptic digest of BSA with very high mass accuracy was obtained using Spiral mode. Using the MASCOT PMF search method, a peptide mass tolerance set as narrow as 10 ppm can lead to highly reliable protein identification with very few false positives. Additionally, the molecular weight of an intact protein was readily obtained by using the JMS-S3000 Linear mode.

References:

[1] US patent US7504620, Japanese patent application JP2006-12782



Applications Note

SpiralTOF-TOF

High-Energy CID Analysis of Bovine Serum Albumin

Introduction

The JMS-S3000 "SpiralTOF™" is a MALDI-TOFMS that uses an innovative SpiralTOF ion optics system to achieve the highest resolution currently available for a MALDI instrument (Fig. 1 and 2). Additionally, this system can be equipped with a TOF-TOF option that can acquire high-energy collision-induced dissociation (CID) product ion spectra for monoisotopically selected precursor ions. The distance to the ion gate is 15 m, more than one order of magnitude longer than that of conventional MALDI TOF-TOF instruments, thus allowing the monoisotopic selection of the precursor ion. The second TOFMS incorporates a re-acceleration mechanism and an offset parabolic reflectron, another innovative ion optical system developed by JEOL. This unique design enables the seamless observation of product ions ranging from very low *m/z* up to that of the precursor ion.

In a previous application note[1], we reported the analysis of bovine serum albumin (BSA) by Spiral and Linear modes. In this work, we show the analytical result for BSA by using the JMS-S3000 "SpiralTOF™" with the TOF-TOF option.

Results and Discussion

As a starting point, the tryptic digest of BSA (tBSA) was analyzed using just the Spiral mode. For the calibration step, a peptide mixture was used as an external mass calibrant, which resulted in an RMS error of 4 ppm. Subsequently, the mass spectrum peak list for this sample was submitted to the MASCOT peptide mass fingerprint

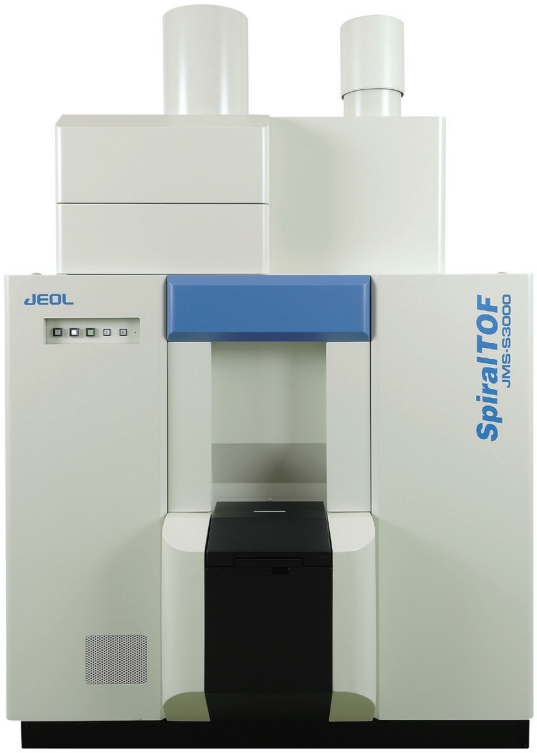


Figure 1. JMS-S3000 SpiralTOF with TOF-TOF attachment.

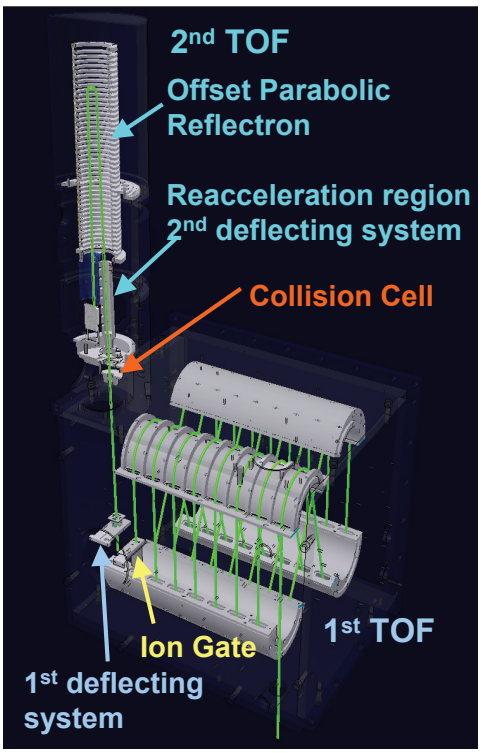


Figure 2. Ion trajectory of Spiral-TOF and TOF-TOF attachment.

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search, and the protein was identified as BSA. These results are in very good agreement with the results obtained previously for Spiral mode.[1]

Next, the 10 highest intensity ions in the tBSA mass spectrum were selected as the precursor ions for automatic measurement using the TOF-TOF option. The product ion spectra for *m/z* 927.5, 1439.8, and 1567.7 shown in Fig.3 had an immonium ion along with the a-ion, d-ion, and w-ion series. These spectra along with the other 7 product ion spectra were then submitted to the MASCOT MS/MS Ion Search, and the protein was identified as BSA (see Fig.4).

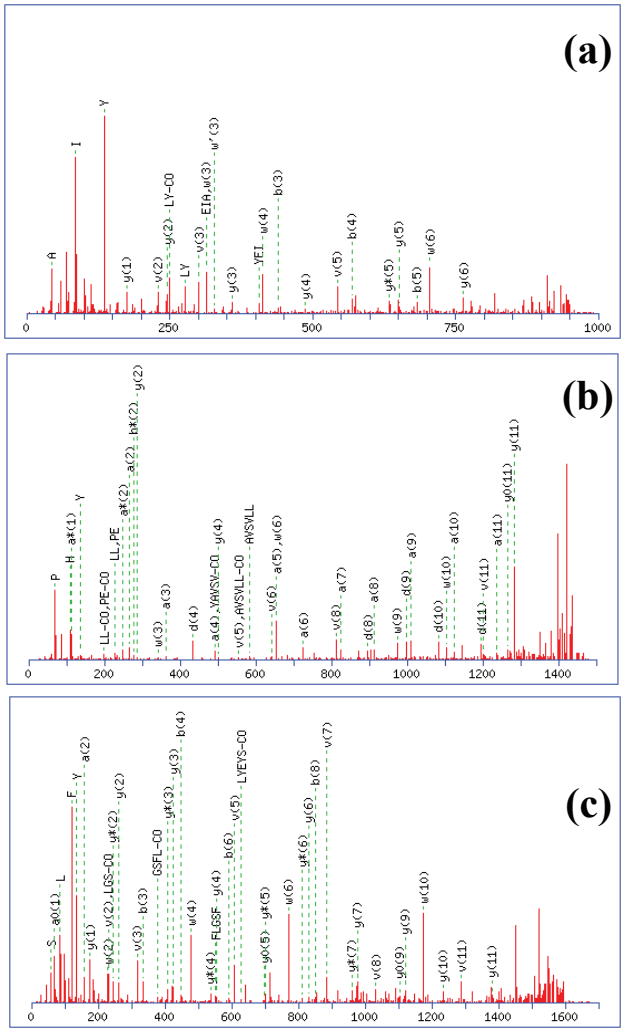


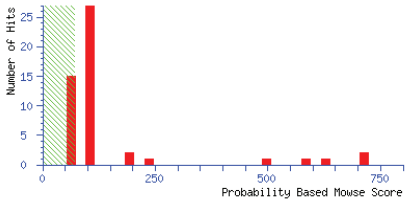
Figure 3. Product ion spectra for *m/z* (a) 927.5, (b) 1439.8, and (c) 1567.7.

Conclusions

The high resolution tBSA mass spectrum measured using Spiral mode was easily identified using the MASCOT peptide mass fingerprint method, even with a peptide mass tolerance set as narrow as 10 ppm (not shown in this note). Additionally, the high energy CID of the 10 highest intensity monoisotopically selected peaks measured using the SpiralTOF-TOF mode produced product ion spectra that readily identified BSA as the protein through the MASCOT MS/MS Ion Search.

Reference

1) www.jeolusa.com/DesktopModules/Bring2mind/DMX/Download.aspx?EntryId=833&PortalId=2&DownloadMethod=attachment



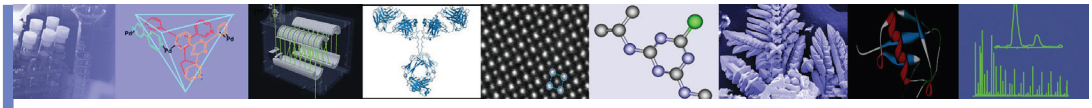
Protein Summary Report

Format As: Protein Summary (deprecated) — Help
Significance threshold p< 0.05 — Max. number of hits AUTO
Standard scoring / MudPIT scoring — Ions score or expect cut-off 0 — Show su
Show pop-ups / Suppress pop-ups — Sort unassigned Decreasing Score — Require
Re-Search All — Search Unmatched

Index

	Accession	Mass	Score	Description
1.	gi|30794280	71309	714	albumin [Bos taurus]
2.	gi|1351907	71279	696	Serum albumin precursor (Allergen Bos d 6) (BSA)
3.	gi|74267962	71221	614	ALB protein [Bos taurus]
4.	gi|229552	68118	576	albumin
5.	gi|76445989	55514	510	serum albumin [Bos indicus]

Figure 4. MASCOT MS/MS Ion search result of tBSA by TOF-TOF mode.



JEOL

Applications Note

SpiralTOF-TOF

Structural Analysis of a High Molecular Weight Peptide

Introduction:

The JMS-S3000 SpiralTOF™ is a MALDI-TOF MS that uses an innovative spiral ion optics system to achieve the highest resolution currently available for a MALDI instrument. Additionally, the JMS-S3000 is available with a TOF-TOF option that acquires high-energy collision-induced dissociation (CID) product-ion spectra for monoisotopically selected precursor ions. In this work, we analyzed a high molecular weight peptide by using the JMS-S3000 SpiralTOF with the TOF-TOF option.

Experimental:

Sample: ACTH18-39 (Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe)

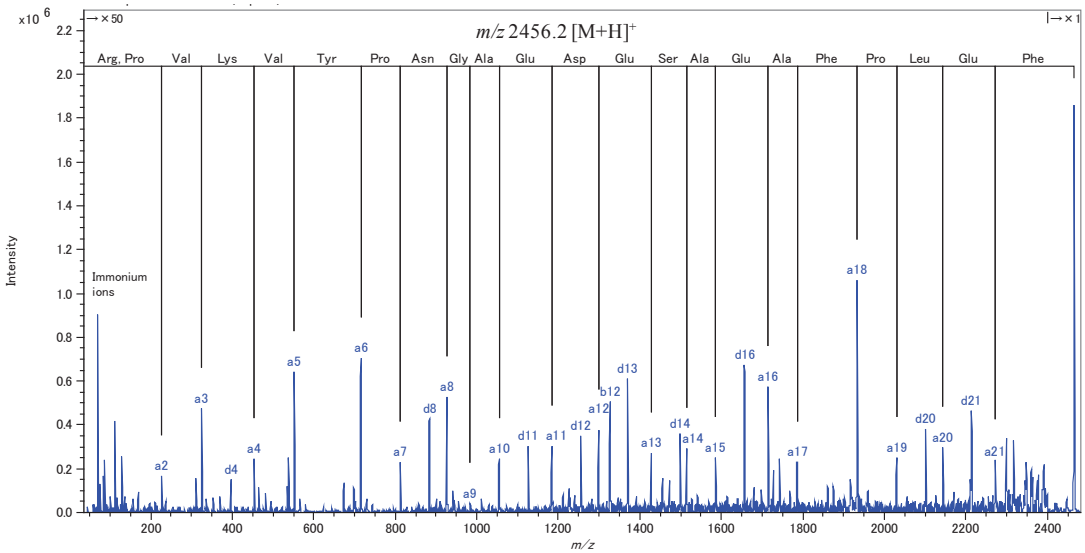
Matrix: α-Cyano-4-hydroxycinnamic acid (CHCA)

Results and Discussion:

The high-energy CID product-ion spectrum for protonated ACTH18-39 $[M+H]^+$ (*m/z* 2456.2) is shown in Fig.1. Each product ion was labeled using the Biemann convention (Fig.2) in which the a-, b-, and d-ion series are fragments generated from the N terminus of the ACTH18-39 molecule. The SpiralTOF MS/MS data in Fig. 1 show that the sequence information is clearly represented by the a-ion series from a2 to a21. The immonium ions (Fig.3) and the d-ion series (produced by fragmentation of the a-ion series) were also observed in the mass spectrum.

Conclusions:

The JMS-S3000 SpiralTOF with the TOF-TOF mode produced high energy CID product ion spectra that clearly identified the sequence for the high molecular weight peptide ACTH18-39.



ACTH18-39: Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe

Figure 1. Product ion spectrum of ACTH18-39 (*m/z* 2456.2, $[M+H]^+$).

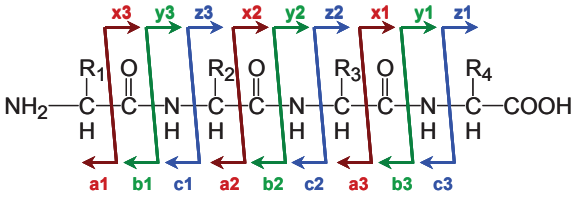


Figure 2. Fragment ion series.

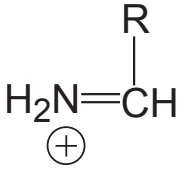
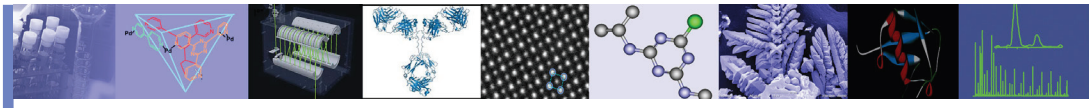


Figure 3. Immonium ion.

MS Tips #175



Applications Note

SpiralTOF™

MALDI-ISD Measurements Using Both the SpiralTOF Mode and the LinearTOF Mode

Introduction

Matrix assisted laser desorption/ionization (MALDI) combined with in-source decay (ISD) is a useful tool for doing top-down sequencing of intact proteins. In this work, we measured and compared the ISD fragment ions generated for several proteins by using both the high resolution MALDI-Spiral mode and the high sensitivity MALDI-Linear mode available on the JEOL SpiralTOF MALDI-MS system.

Experimental

Myoglobin and Bovine serum albumin (BSA) protein samples were separately dissolved into 0.1% trifluoroacetic acid aqueous with the concentration fixed at 10 pmol/µL. 1,5-diaminonaphthalene (DAN), which can provide good S/N for ISD fragment ions [1], was used as the MALDI matrix. The DAN matrix was dissolved to 0.1% trifluoroacetic acid aqueous/ 50% acetonitrile with the matrix concentration fixed at 10 mg/mL. Subsequently, the matrix and the sample solutions were mixed 1/1 (v/v), and then 1 µL of each solution was deposited and dried on the MALDI target plate. Afterwards, each sample was analyzed in triplicate on the JEOL JMS-3000 SpiralTOF by using both the SpiralTOF mode and the LinearTOF mode.

Results

The Myoglobin and BSA ISD mass spectra for both the LinearTOF and SpiralTOF measurements are shown in Figures 1 and 2, respectively. Both sets of ISD spectra were dominated by the c-ion series. The Linear mode mass spectra showed higher sensitivity overall, especially for ions over *m/z* 5,000 (shown in the *m/z* 4000-7000 zoomed regions). However, as expected, the Linear TOF did not provide full isotopic separation of these c-ions (see insets for the myoglobin c35 and BSA c33 ions). Conversely, the Spiral mode measurements fully resolved these ions and their associated isotopes, as shown in the Figures 1b and 2b insets, respectively, but had less sensitivity at higher *m/z* values.

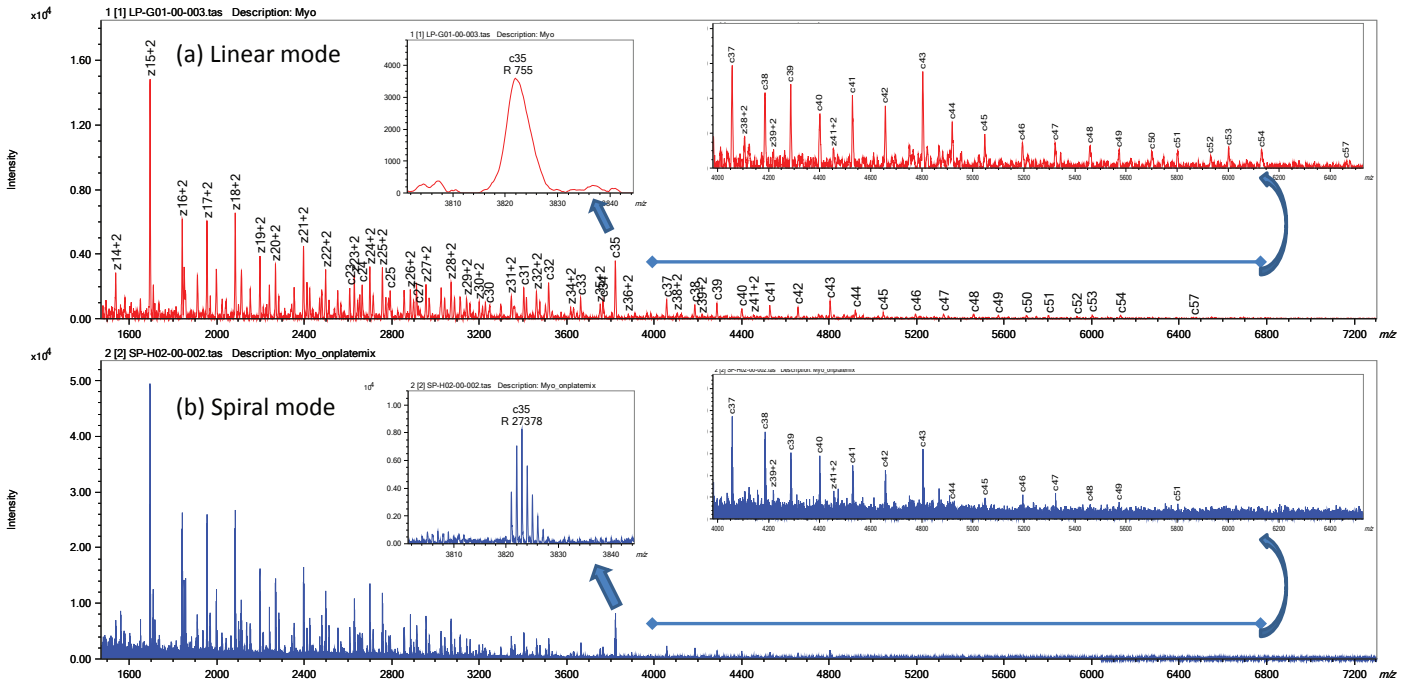


Figure 1. ISD Spectra of myoglobin (MW: 16,952Da) using (a) LinearTOF mode, (b) SpiralTOF mode.

MS-060913

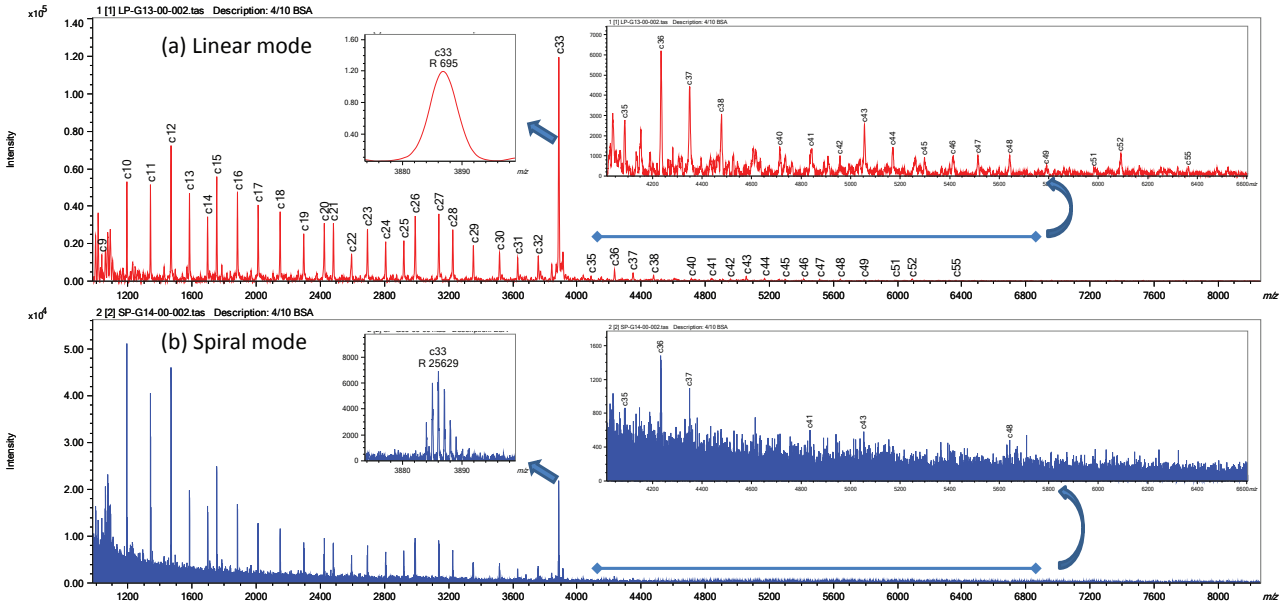


Figure 2. ISD spectra of BSA (MW: 66,430Da) using (a) LinearTOF mode, (b) SpiralTOF mode.

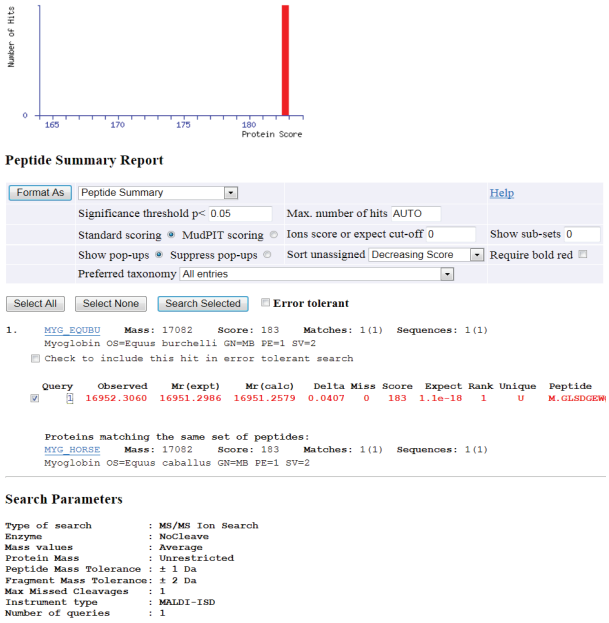


Figure 3. Mascot search result for the myoglobin sample.

A Mascot MS/MS Database Search using the LinearTOF data identified each sample as myoglobin and BSA, respectively. As an example, Figure 3 shows the Mascot database search result for the myoglobin sample. These results showed that, despite the fact that the ISD Linear data does not contain exact masses and does not provide isotopic separation of the ions, the data can still be used with a database search function like Mascot to identify proteins.

Conclusion

In this work, we showed a brief study in which the ISD measurements for standard proteins were measured by using the SpiralTOF mode and the LinearTOF mode. The SpiralTOF mode provides high mass accuracy and fully separated isotopic ions while the LinearTOF mode provides higher sensitivity, particularly for ions in the higher *m/z* region. Additionally, the LinearTOF data can be used in conjunction with a database search analysis to identify proteins.

Reference

[1] Issey Osaka, Mami Sakai, Mitsuo Takayama, 5-Amino-1-naphthol, a novel 1,5-naphthalene derivative matrix suitable for matrix-assisted laser desorption/ionization in-source decay of phosphorylated peptides, Rapid Communications in Mass Spectrometry, Volume 27, Issue 1, pages 103–108, 15 January 2013.

Structural analysis of phospholipids in egg yolk using JMS-S3000 “SpiralTOF™” with TOF-TOF option

Product used : Mass spectrometry (MS)

Phospholipids are one of the major components of egg yolk. In this study, we extracted phospholipids from the egg yolk and tried to analyze their structures with the TOF-TOF option of the JMS-S3000 SpiralTOF™.

Measurement conditions

First, the egg yolk was dissolved in a mixed solution of chloroform, methanol, and water in order to separate lipids from water-soluble components such as proteins. After centrifugation, the chloroform/methanol layer was taken out, diluted with methanol, mixed with the matrix solution at a ratio of 1:1, and spotted onto the target plate.

Measurement results and summary

Fig. 1 shows the mass spectra of positive ion mode and negative ion mode measured in SpiralTOF mode. Judging from the *m/z* values of the observed ions, it is thought that phosphatidylcholines (PCs) were mainly observed in the positive ion mode, and phosphatidylinositols (PIs) were mainly observed in the negative ion mode. To confirm this, we measured the product ion mass spectra in TOF-TOF mode. In MSTips No.186, we reported the product ion mass spectrum of $[M+H]^+$ of a standard sample 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (PC(16:0, 18:1)) with the total composition of PC(34:1) The $[M+H]^+$ of PC(34:1), which seems to have the same composition as the standard sample, was observed on the mass spectrum of the egg yolk sample. We selected this ion as a precursor ion, measured the product ion mass spectrum, and examined the structural similarity by comparing the spectra (Fig.2). In the negative ion mode, PI (38:4) $[M-H]^-$ with strong intensity was selected as the precursor ion. The product ion mass spectrum was measured (Fig. 3). Looking at the spectrum in Fig. 2. However, there is a slight difference, the same result as that of the standard sample was obtained, and the selected precursor ion was confirmed to be derived from PC(16:0,18:1). Next, looking at the spectrum in Fig. 3, each peak can be assigned as shown in Fig. 4, which is presumed to be PI(18:0,20:4).
As described above, it is possible to perform a structural analysis of phospholipids by performing measurements using the TOF-TOF option.

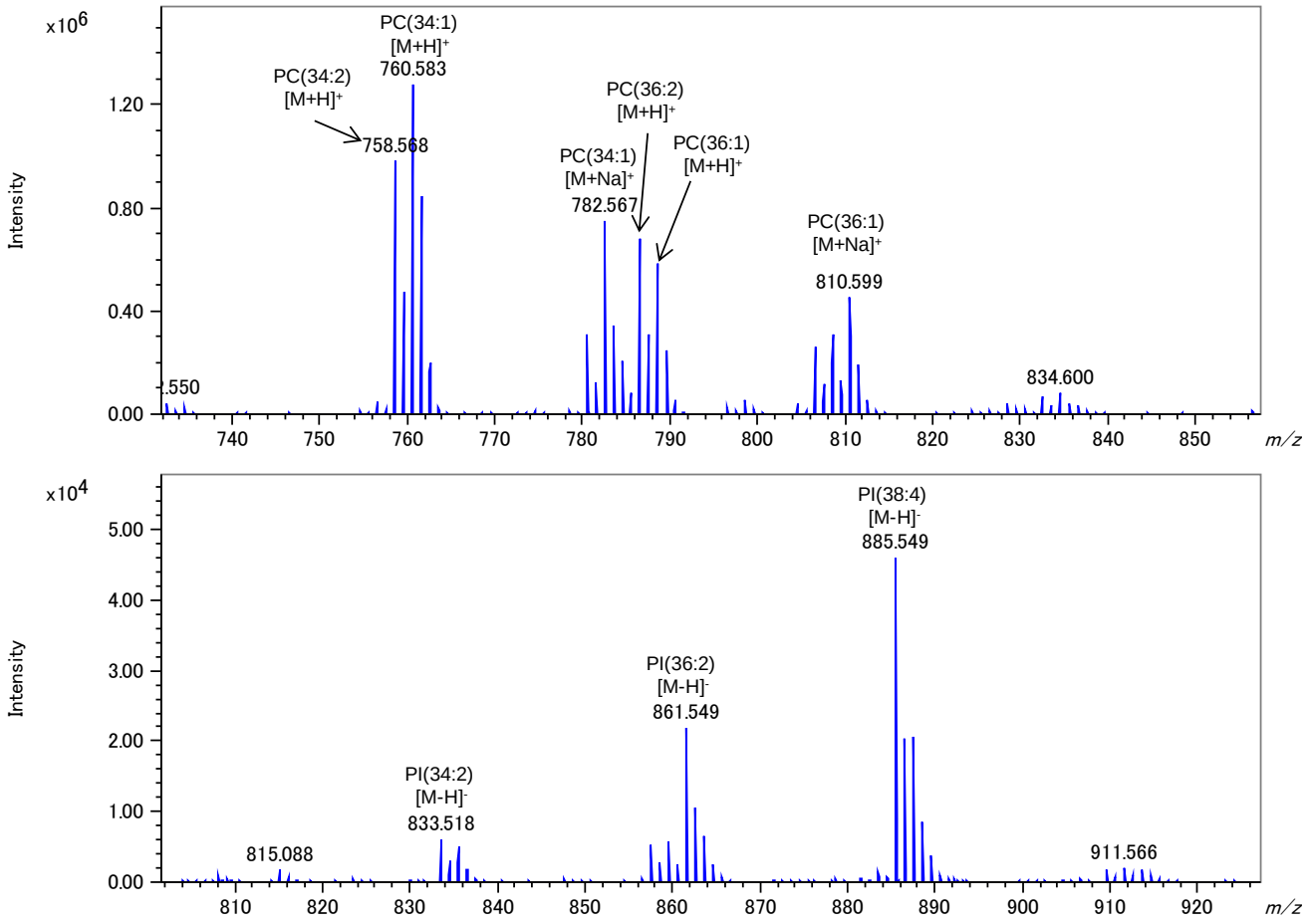


Fig.1 Mass spectra of phospholipids from egg yolk (top:positive ion mode, bottom negative ion mode).

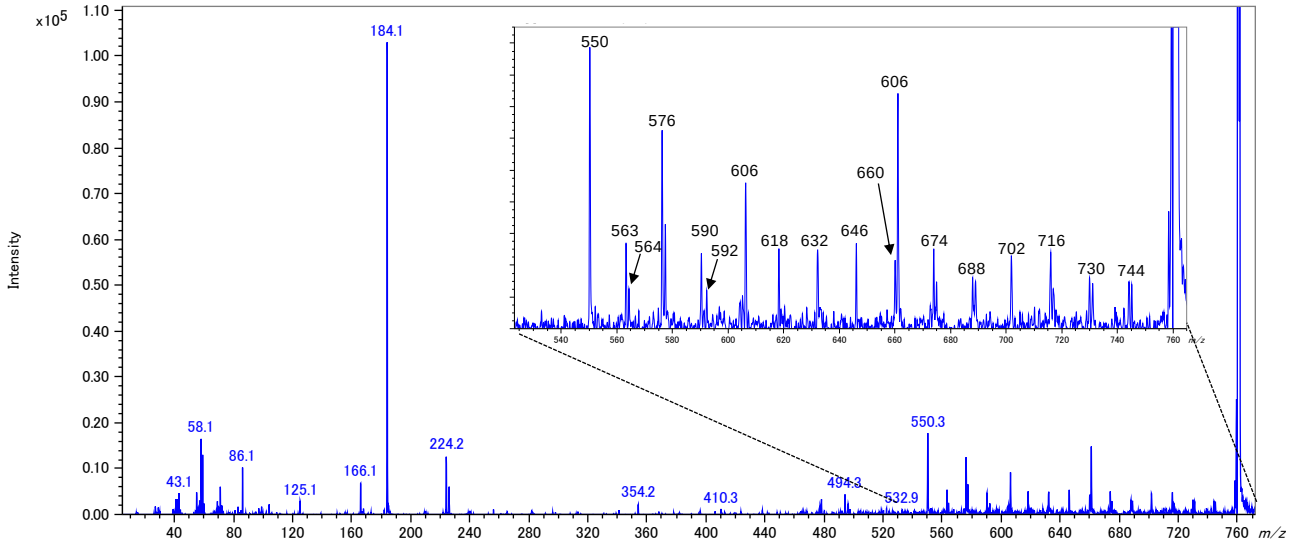


Fig.2 Product ion spectrum of PC(34:1) $[M+H]^+$

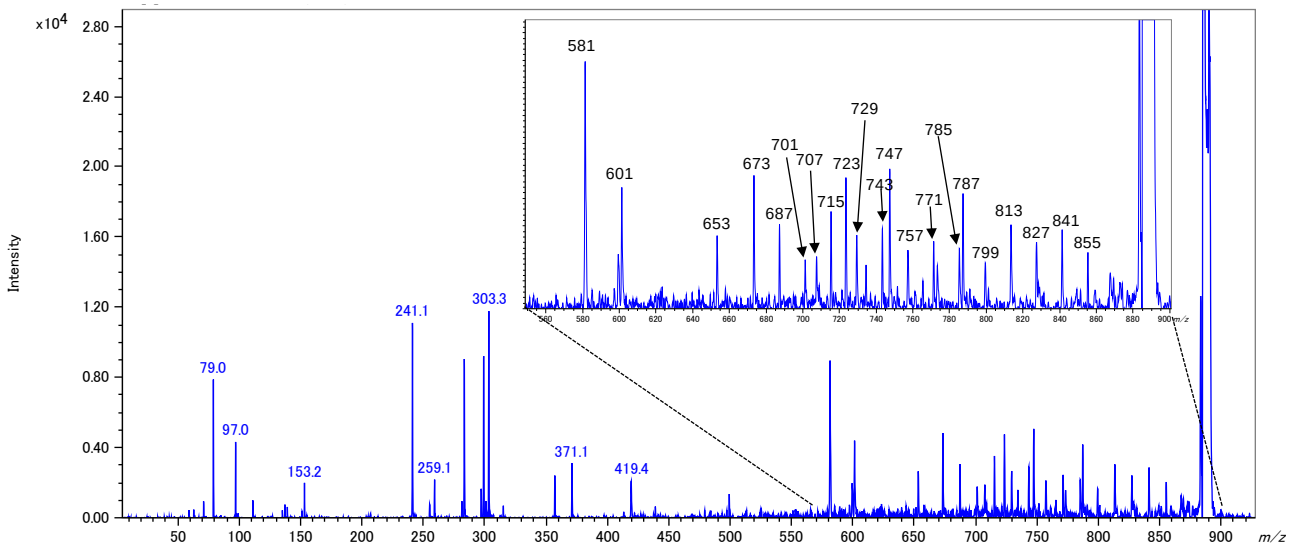


Fig.3 Product ion spectrum of PI(38:4) $[M-H]^-$

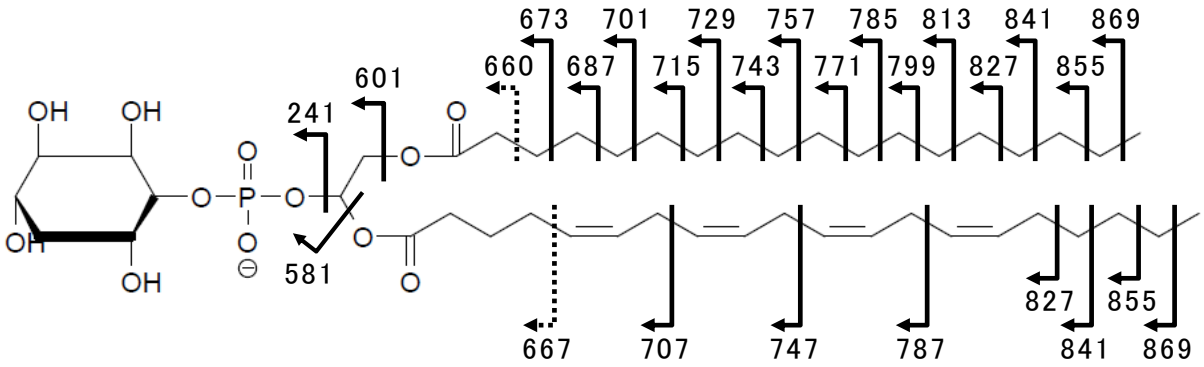


Fig.4 Peak assignment of product ion spectrum of PI(38:4) $[M-H]^-$

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Applications note

MS
MSTips No. 364
MALDI-TOFMS Application

Analysis of oligonucleotides using JMS-S3000 "SpiralTOF™-plus 2.0"

Product used : Mass spectrometer (MS)

Introduction

A nucleotide is a compound in which a phosphate group is bound to a nucleoside consisting of a base and a sugar and is a building block of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acid drugs are attracting attention as therapeutic agents for diseases that have been difficult to treat, and as of October 2022, 16 nucleic acid drugs have been approved in Japan, U.S.A., or EU[1]. In recent years, synthesized oligonucleotides have been utilized as nucleic acid medicines. Molecular weight confirmation of synthesized oligonucleotides is important for the quality control of pharmaceuticals. In this report, we used the synthesized oligonucleotide (Table 1) as a sample and measured it with a MALDI-TOFMS.

Table1) The base sequence of synthesized oligonucleotide and *m/z* value.

Sequence	Lowest *m/z* value of $[M+H]^+$	Average *m/z* value of $[M+H]^+$
5'-CGCTAAGTACGCAATGGGCC-3'	6125.0710	6127.9928

Measurement conditions

The matrix 3-HPA (3-Hydroxypicolinic acid) was prepared to 20 mg/mL 50% AcN (20 mM diammonium citrate aqueous solution). A 50 µM aqueous solution of the synthesized oligonucleotide was used as a sample. After mixing the matrix solution and the sample solution at 5:1 (v/v), they were spotted on a target plate, dried in air, and measured in Linear TOF mode (positive ion mode) and SpiralTOF mode (positive ion mode).

Results and summary

Fig. 1 shows the measurement results in Liner and Spiral TOF modes. In both modes, protonated molecules of oligonucleotides were observed as the most intense peaks, and sodium ion adducts were also observed. In the Linear TOF mode, it is difficult to resolve the isotope peaks due to the low mass resolution, but in the SpiralTOF mode, a mass resolution of about 40,000 was obtained, and the isotope pattern was clearly observed. Comparing the simulated isotope pattern (Fig. 2a) and the measured mass spectrum (Fig. 2b), the isotopic peak patterns are well agreed. The mass error of the monoisotopic peak was confirmed to be -6.4 mDa (-1.0 ppm) by the external standard method.

As described above, JMS-S3000 "SpiralTOF™-plus 2.0" was shown to be a very effective analytical tool for nucleic acid analysis.

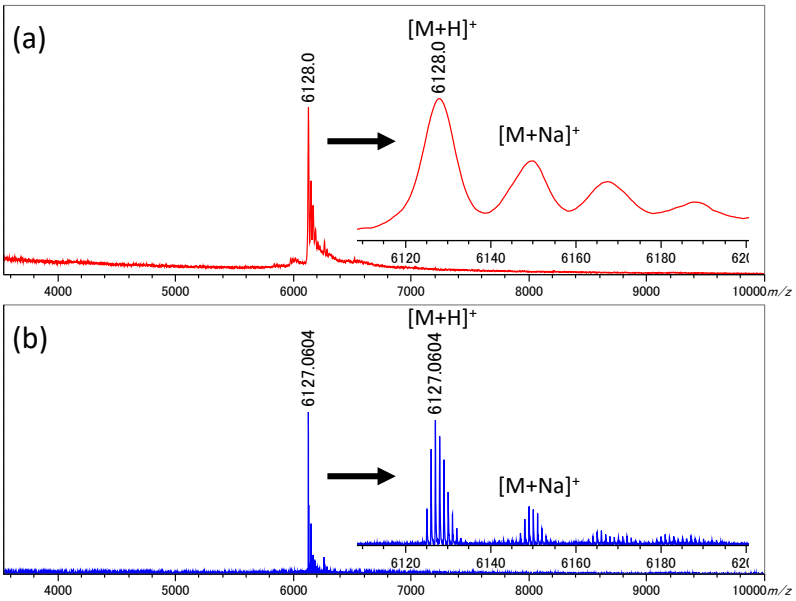

Fig. 1 Mass spectra of LinearTOF mode(b) and SpiralTOF mode(a) .

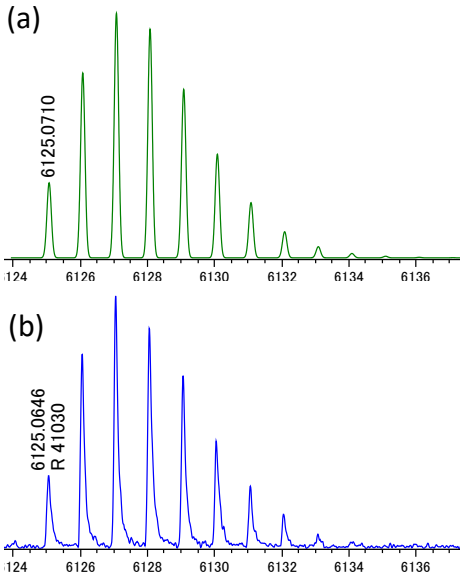

Fig. 2 Comparison of theoretical isotope pattern(a) and obtained isotope pattern of $[M+H]^+$(b).

Reference

1) Web site of the division of molecular target and gene therapy products, national institute of health sciences, Japan (https://www.nihs.go.jp/mtgt/pdf/section2-1.pdf)

Acknowlegement

Sample courtesy of Prof. Masumi Taki, the University of Electro-Communications

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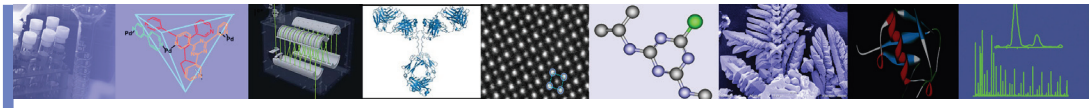

Applications Note

SpiralTOF-TOF

High-energy CID Mass Spectrometry of Oligosaccharides

Introduction:

Matrix assisted laser desorption ionization (MALDI) is a powerful and useful ionization technique that is commonly used for the analysis of biomolecules such as oligosaccharides. There are many applications of oligosaccharides in which various ionization techniques and mass spectrometers were used for their analysis [1]. In particular, tandem mass spectrometry techniques are often used to sequence these molecules.

Recently, JEOL developed a new tandem TOF-TOF instrument coupled with MALDI that is called the SpiralTOF. The 1st TOF consists of 4 toroidal electric sectors that fold a 17 meter flight path into a one meter box. This design provides several unique advantages for TOF-TOF analysis. The 2nd TOF has (a) 20 kV high-energy CID, (b) monoisotopic precursor ion selection, and (c) no PSD ions in the product ion mass spectrum.

In this study, we analyzed several oligosaccharides by using the JMS-S3000 SpiralTOF-TOF tandem mass spectrometer system.

Experimental:

All oligosaccharides (Laminaritetraose, Stachyose, α-Cyclodextrin, β-Cyclodextrin, γ-Cyclodextrin) were commercially available items that were used without further purification. Each oligosaccharide standard solution was dissolved in water. 2,5-Dihydroxybeozoic acid (DHB) was dissolved in 40% ethanol at a concentration of 10 mg/mL. Next, the oligosaccharides standard solution and matrix solution were mixed together 1:1 by volume. Afterwards, 0.5 µL of this mixture was placed on the MALDI target plate. Finally, the dried sample was measured using the JMS-S3000 SpiralTOF-TOF.

Results:

The A, B and C fragment ions for oligosaccharides are labeled as the non-reducing terminal ends while the X, Y and Z fragment ions are labeled as the reducing terminal ends. The nomenclature fragmentation pathway of oligosaccharides by tandem MS is shown in Figure 1[modified from Reference 2].

In this study, we did not do further purification for all oligosaccharide standards, matrix and solvents. As a result, the sodiated molecules were the most intense peak in each positive-ion MALDI mass spectrum (see Figure 2). Therefore, these sodiated molecules were selected as the precursor ions for the SpiralTOF-TOF analysis. MALDI TOF-TOF spectra of Laminaritetraose and Stachyose are shown in Figure 3. Laminaritetraose is a tetrasaccharide that consists of four β-D-glucose units that are linked as Glc(β1→3)Glc(β1→3)Glc(β1→3)Glc. Stachyose is also a tetrasccharide that consists of two α-D-galactose units, one α-D-glucose unit, and one β-D-fructose unit that are linked as Gal(α1→6)Gal(α1→6)Glc(α1→2β)Fru. These isomers showed significantly different mass spectral patterns. B and Y fragment ions were generated from the glycosidic bond cleavage were observed as the dominant components in each MALDI TOF-TOF spectrum. The fragmentation scheme for Laminaritetraose is shown in Figure 4. High-energy CID fragmentation occurs within a shorter time-scale than low-energy CID, which means that it provides a cross-ring cleavage that gives useful structural information about the oligosaccharides and their glycoconjugates. This cross-ring cleavage was also observed for both oligosaccharides, as indicated by the presence of the fragment ions m/z 599, 569, 555, etc. (see Figure 3). All of this information is essential for determining the structure of each oligosaccharide and differentiating structural isomers from each other.

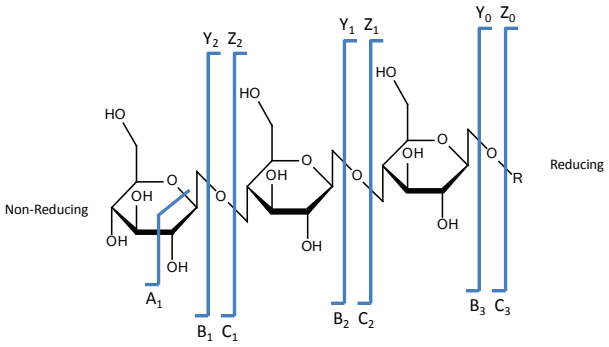

Figure 1. Fragmentation pathways.

Cyclodextrins are cyclic oligosaccharides consisting of five or more α-D-glucose units that are linked as Glc(α1→4)Glc. The MALDI TOF-TOF spectrum for each Cyclodextrin is shown in Figure 5. In each case, the B ion series from glycosidic bond cleavage were the dominant ions observed in the TOF-TOF spectra. Additionally, a number of fragment ions were observed that were the result of cross-ring cleavage.

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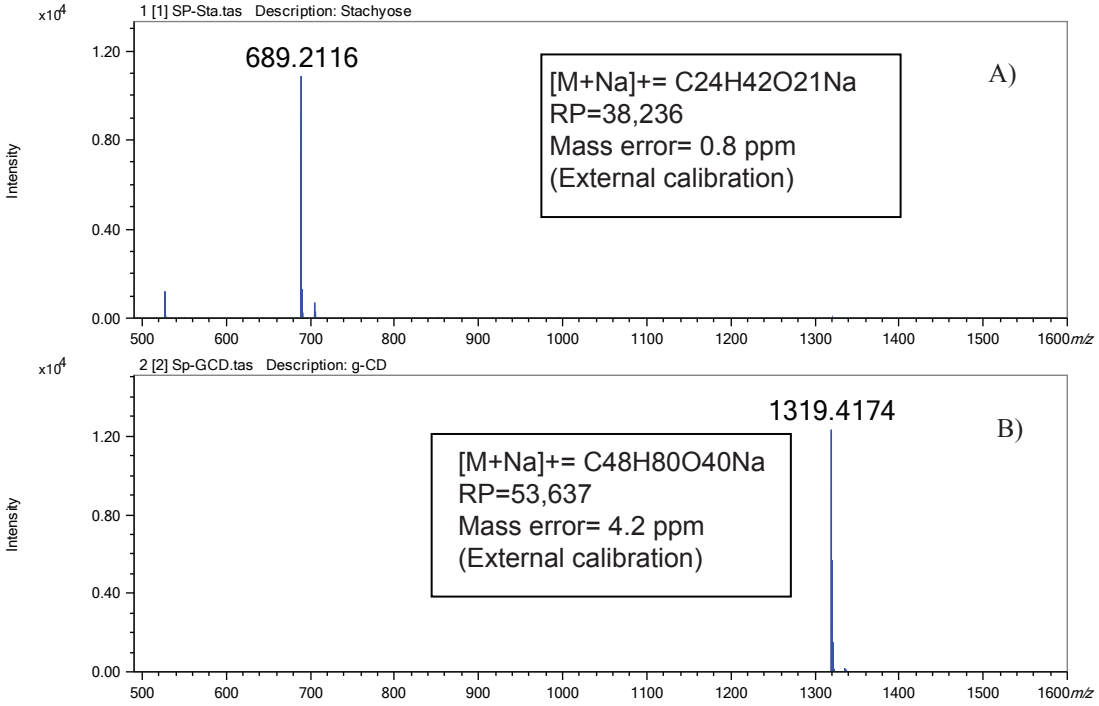


Figure 2. MALDI TOF spectra. A) Stachyose, B) γ-Cyclodextrin

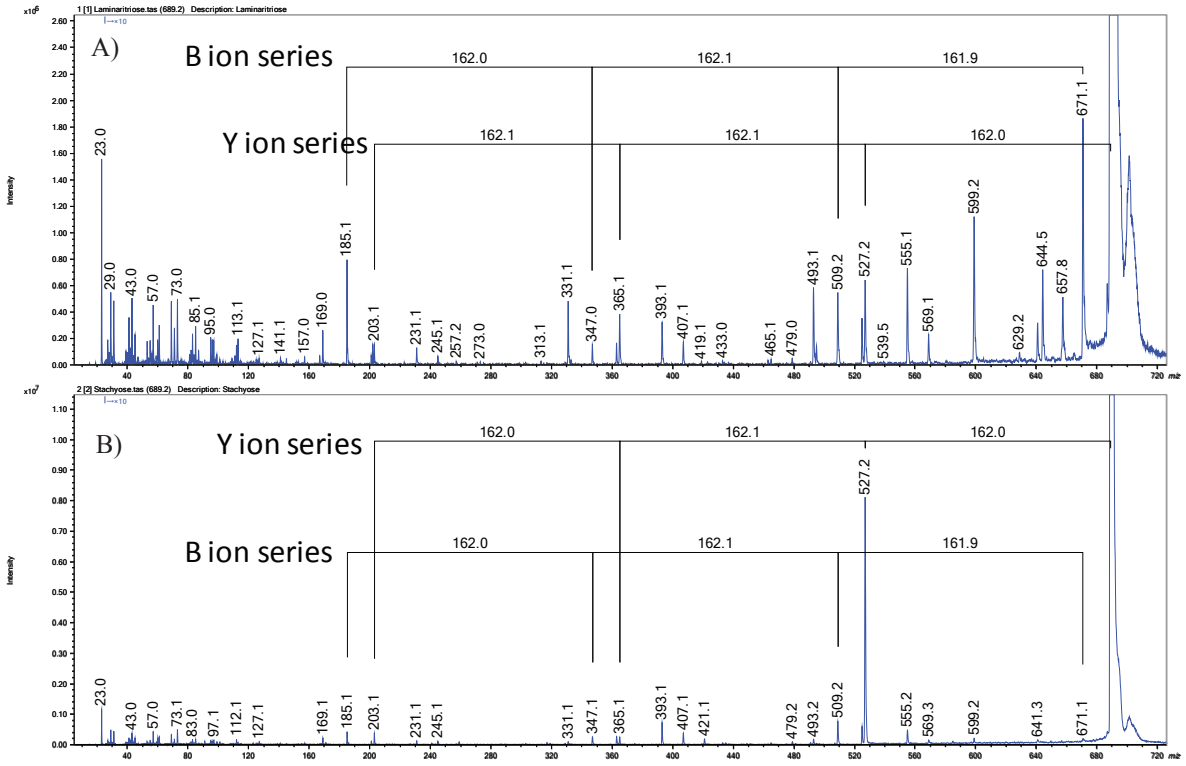


Figure 3. MALDI TOF-TOF spectra. A) Laminaritetraose, B) Stachyose

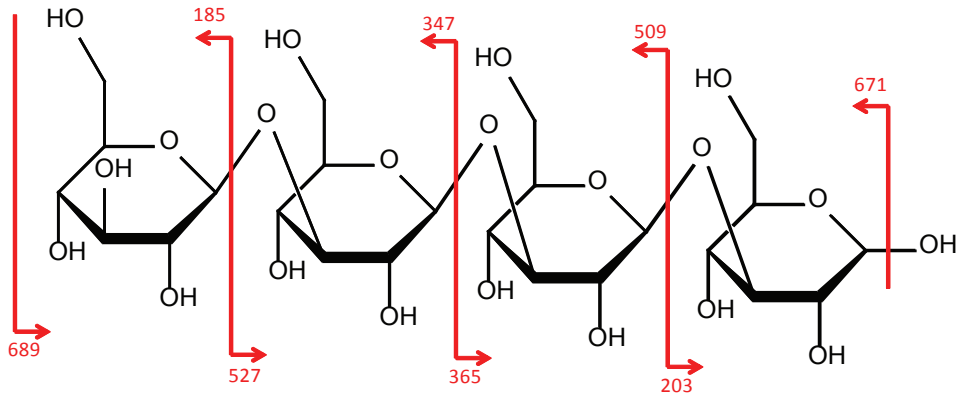


Figure 4. The glycosidic bond cleavage to generate B and Y ion series for Laminaritetraose

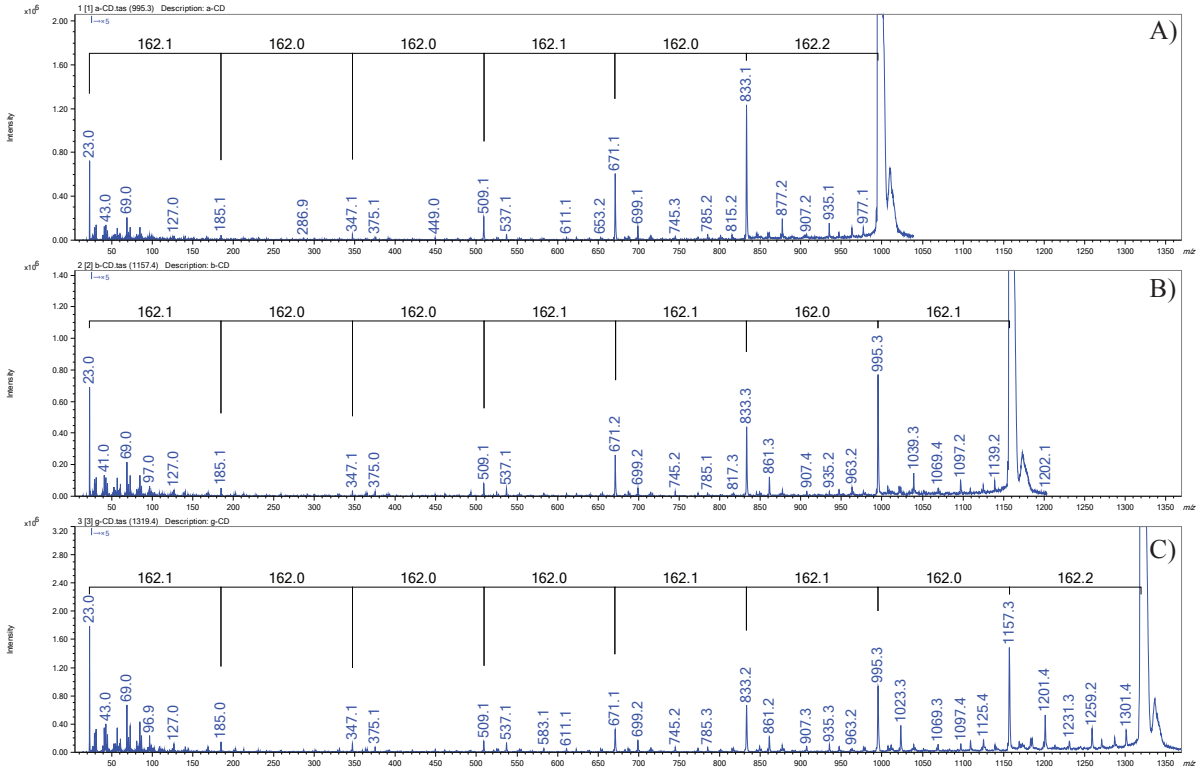


Figure 5. MALDI TOF-TOF spectra. A) α-Cyclodextrin, B) β-Cyclodextrin, C) γ-Cyclodextrin

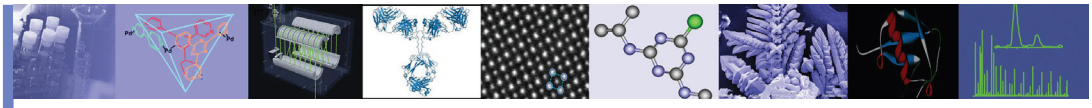
Conclusion:

In this work, we showed a brief study in which the high-energy CID measurements for several oligosaccharides were measured by using the SpiralTOF-TOF. The B and Y ion series were observed as the main components in each TOF-TOF spectrum. Furthermore, we could also see a number of fragment ions that were generated from cross-ring cleavage, which was helpful in differentiating structural isomers. These results show that high-energy CID coupled with MALDI provides a good platform for determining oligosaccharide structural information that will not generally be available when analyzing these compounds by other mass spectrometry techniques.

Reference:

[1] Joseph Zaia, Mass Spectrometry of Oligosaccharides, ***Mass Spectrometry Reviews***, Volume 23, Issue 3, pages 161–227, May/June 2004

[2] Domon B, Costello CE. 1988b. A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra ofglycoconjugates. ***Glycoconjugate J*** 5:397–409.



Applications Note

SpiralTOF-TOF

Analysis of the Natural Organic Compound YTX by Using TOF-TOF Option

Introduction

Yessotoxin (YTX) is one of the substances that cause food poisoning when humans consume toxic shellfish. The structure of this compound has been previously analyzed by high-energy collision induced dissociation (CID) using a traditional four sector tandem double focusing mass spectrometer.[1]

In this work, we analyzed YTX by using a JMS-S3000 SpiralTOF™ equipped with the optional TOF-TOF analyzer to generate a high energy CID product ion spectrum.

Results and Discussion

Fig. 1 shows the YTX mass spectrum acquired using polyalanine as an external calibrant. The spectrum shows a peak at *m/z* 1061.513, which is suspected to be the monoisotopic ion $[M-2Na-SO3+H]^-$ (calculated value 1061.609). There was also a $[M-Na]^-$ peak observed for YTX at *m/z* 1164.450 (calculated value 1163.548). Among these peaks, the product ion spectrum was acquired from $[M-2Na-SO_3+H]^-$, which had been previously analyzed using a traditional four sector tandem double focusing mass spectrometer.1 Fig. 2 shows the high-energy CID results generated by the SpiralTOF-TOF. The negative charge was fixed to the sulfate ester end of the molecule, causing charge remote fragmentation (CRF) to occur. As a result, the spectrum showed peaks that systematically reflected the structure of YTX, as shown in Fig. 3. This data closely resembles the results obtained using a traditional four sector MS/MS system.[1]

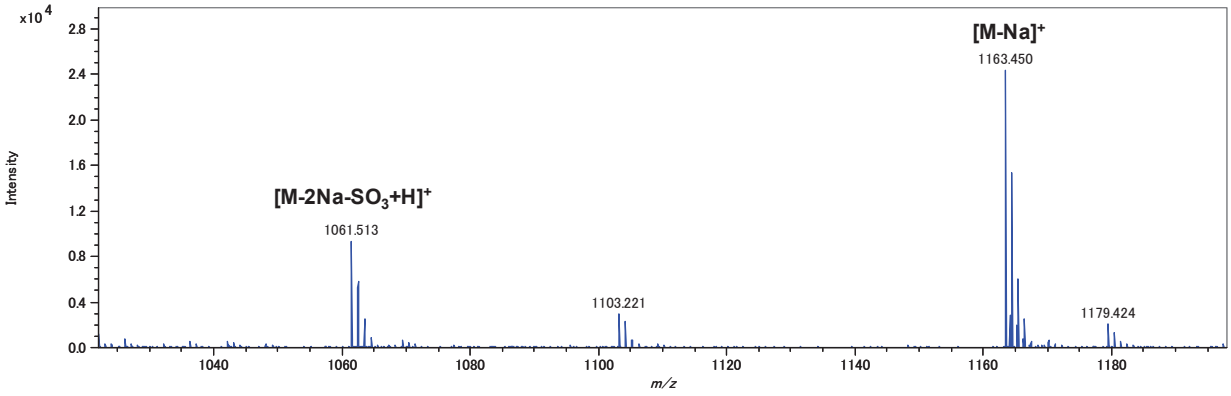


Figure 1. Mass spectrum of YTX.

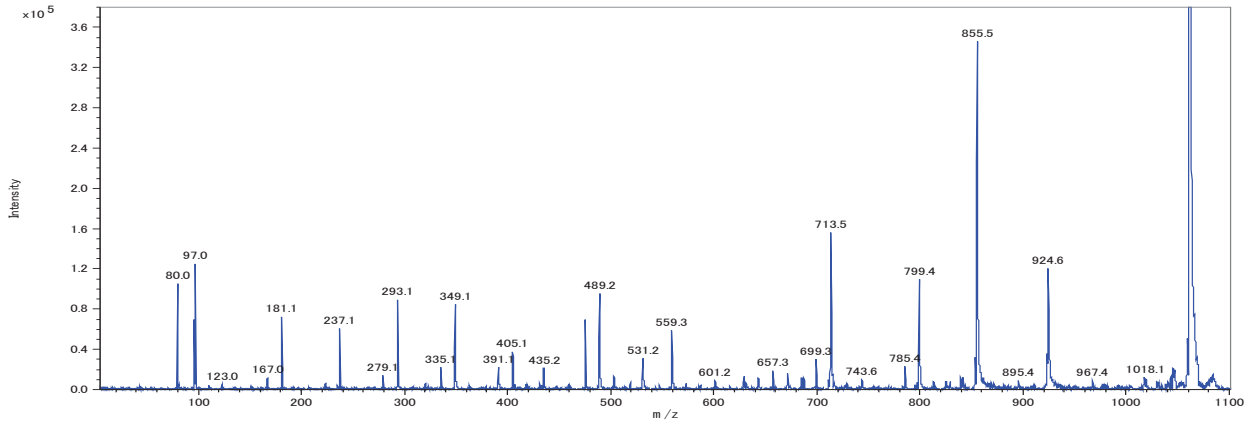


Figure 2. Product ion spectrum of $[M-2Na-SO_3+H]^-$

MS-050813A

Conclusions

As this data demonstrates, high energy CID analysis using SpiralTOF-TOF clearly detected the peaks resulting from CRF, Which enabled analysis of samples that are complex in structure such, as natural organic compounds.

Acknowledgement

We wish to express our thanks to Prof. Michio Murata of the Department of Biomolecular Sciences, Department of Physics, Graduate School of Science, Osaka University, for providing the YTX samples.

Reference

[1] H. Naoki, M. Murata, T. Yasumoto, Rapid Communication of Mass Spectrometry 7 (1993) 179

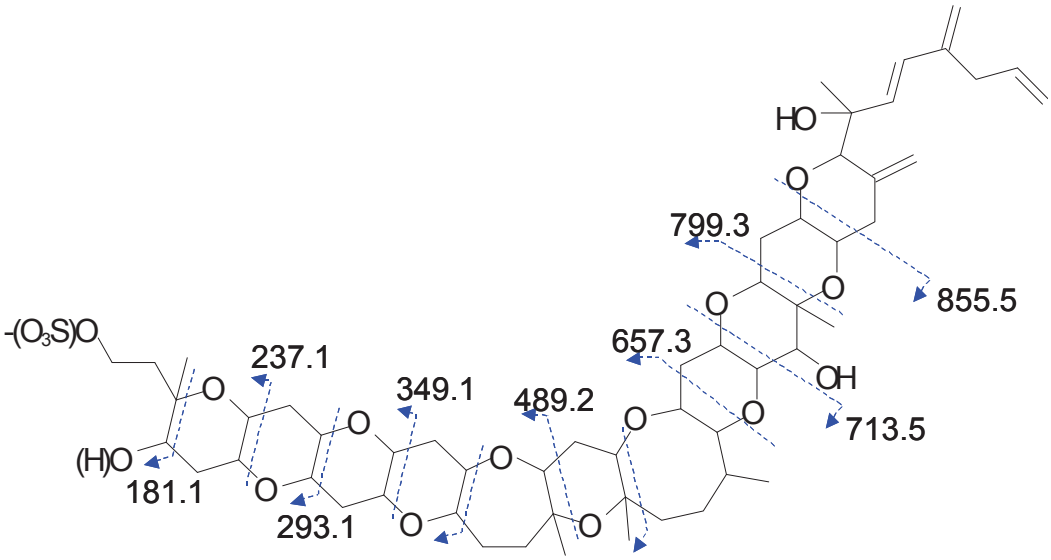


Figure 3. Structure of YTX and fragmentation pattern.

Applications note

MS
MSTips No. 370
MALDI-TOFMS Application

High mass-resolution MS imaging using JMS-S3000 "SpiralTOF™" and statistical data analysis

Product used : Mass spectrometer (MS)

Imaging mass spectrometry (MSI) using matrix-assisted laser desorption/ionization (MALDI) is a technique for visualizing the distribution of organic compounds on a sample surface. Applications are being expanded mainly for proteins, peptides, lipids, drugs, and their metabolites on the surface of frozen tissue sections. MALDI-MSI scans the laser irradiation position two-dimensionally on the sample surface and acquires a mass spectrum at each laser irradiation position. By analyzing this series of mass spectra with two-dimensional positional information, the distribution of organic compounds with arbitrary molecular weights on the sample surface can be drawn as an extracted mass image.
The JMS-S3000 SpiralTOF™ is a time-of-flight mass spectrometer (TOFMS) with patented spiral trajectory ion optics. Because it has a flight distance of 17m, which is longer than ordinary reflectron TOFMS, high mass resolution can be achieved even in MSI, where the surface condition of the sample is often uneven. In addition, the ion optical system is composed of sector electric fields, which makes it possible to remove post-source decay (PSD) ions, and it is also possible to detect trace components near the baseline with high mass resolution. Therefore, even in MALDI-MSI, it is possible to separate compounds with the same nominal mass but different exact masses (isobars) and draw a clear distribution of the compounds. In this report, we report the statistical analysis of high-mass resolution MALDI-MSI data of various lipids in biological samples.

Measurement conditions

Mouse brain frozen tissue sections were used as samples. The matrix was DHB, and its solution was sprayed with an airbrush. MS imaging data were acquired in SpiralTOF mode (positive ion mode) with a pixel size of 40µm.

Measurement results

Figure 1 shows the average mass spectrum obtained by MALDI-MSI. In the following, PC is phosphatidylcholine, PE is phosphatidylethanolamine, and GalCer is galactosylceramide. Figure 1a also shows mass images of two peaks, PC(32:0) and PC(34:1), which have high ion intensities. On the other hand, as can be seen from the average mass spectrum, most of the observed peaks have intensities less than 10% of the base peak. Figure 1b shows an enlarged image of m/z 820-823. Peaks with a difference of about 0.1u are observed every 1u in the enlarged view. These peaks are derived from PE (36:2), PC (36:4), PE (36:1), and GalCer (d18:1/22:0), respectively, and their localization in the frozen tissue section is also different. Using high mass-resolution MALDI-MSI makes it possible to separate compounds with small mass differences and obtain accurate localization information.

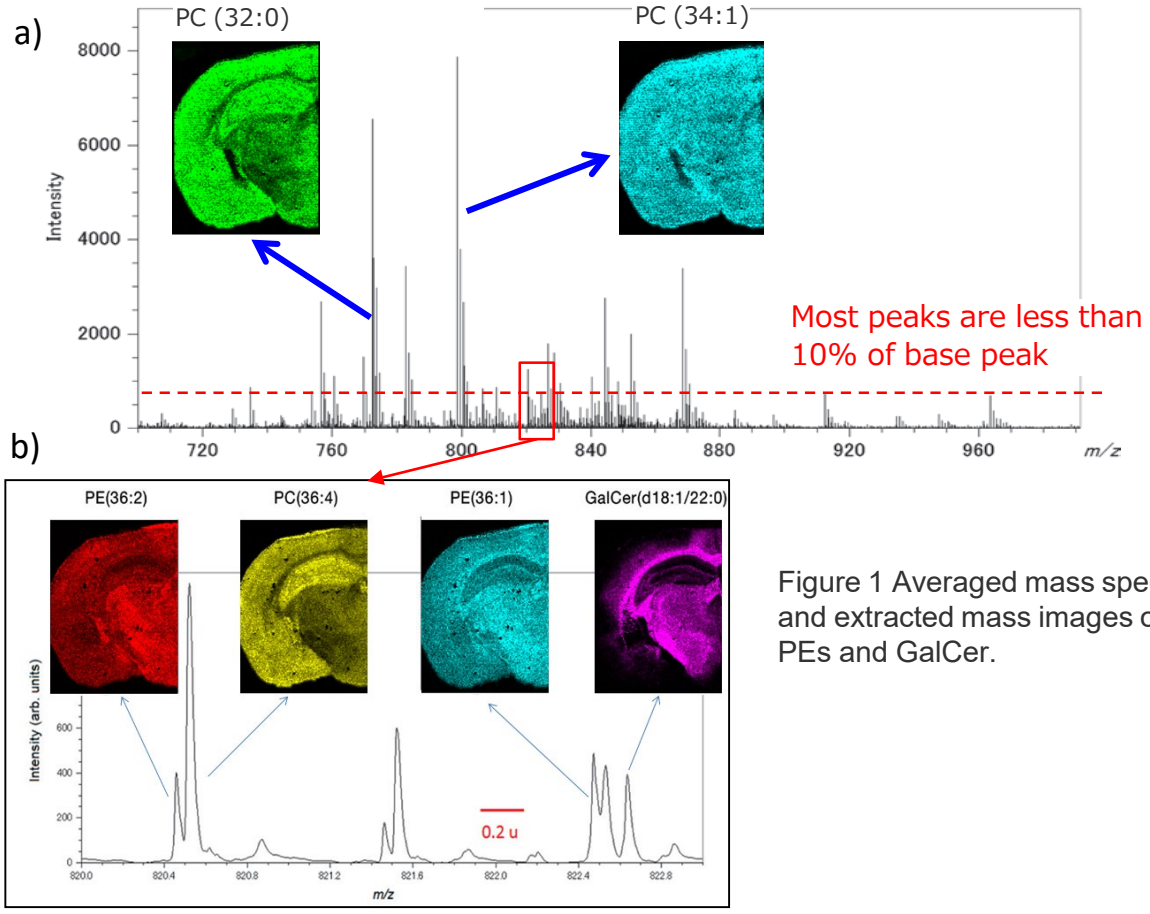
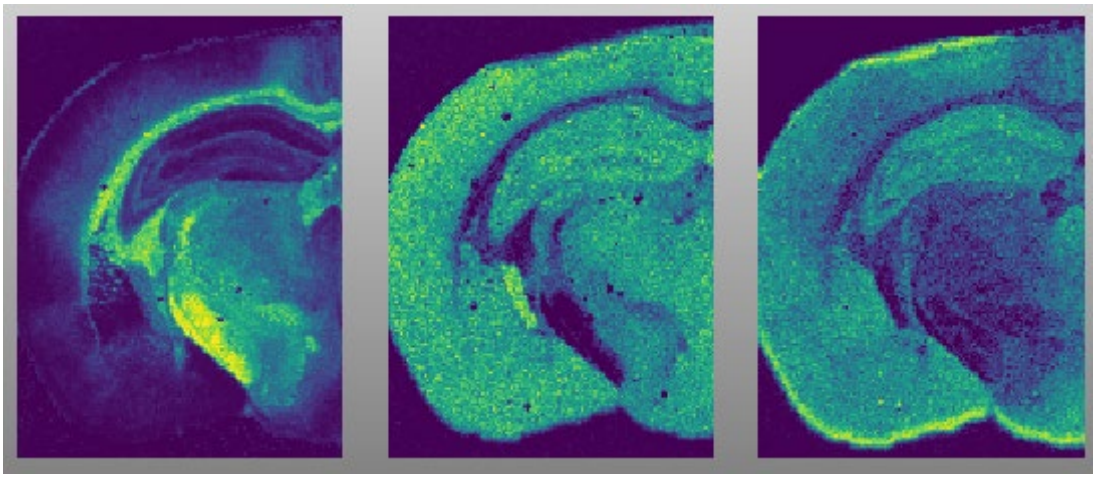


Figure 1 Averaged mass spectrum and extracted mass images of PCs, PEs and GalCer.

Statistical analysis of MALDI-MSI data

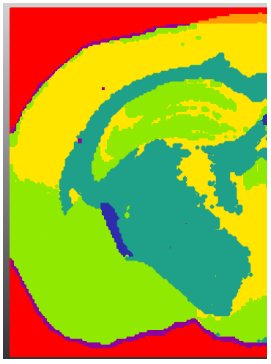
Statistical analysis is also effective for grasping the whole picture in the analysis of mass imaging data with many peaks. SCiLS Lab MVS, Version 2020b Premium3D was used for statistical analysis. MS imaging data obtained by SpiralTOF™ was imported into this software after being converted to imzML format. First, Figure 2 shows the results of pLSA (Probabilistic latent semantic analysis) analysis. Looking at Components 1 to 3, we can see that characteristic localization information can be obtained in the mass imaging data. In this way, it is possible to capture the characteristics of the mass spectral pattern for each pixel of the high mass-resolution MALDI-MSI data and find characteristic regions. Next, Figure 3 shows the results of the segmentation analysis. In segmentation analysis, characteristic parts can be color-coded by statistical methods. In addition, it is possible to create a mass spectrum for each color-coded region, making it easier to search for characteristic components for each region.



Component1 Component2 Component3

Figure 2 pLSA analysis of the high mass-resolution MALDI-MSI data

Segmentation result



Mass spectrum of each segment at *m/z* 820-826

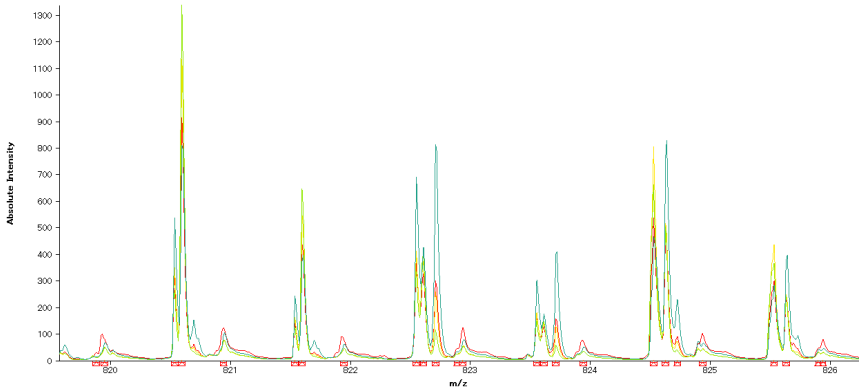


Figure 3 Segmentation of the high mass-resolution MALDI-MSI data

Summary

SpiralTOF™ enables the analysis of trace components even in the low molecular weight region due to its high mass-resolution and removal of PSD ions. This feature is also valid for MALDI-MSI. In applications where many peaks are observed, such as lipid analysis in frozen tissue sections, combining high-mass-resolution MALDI-MSI with statistical techniques enables more efficient analysis.

The data were acquired in a joint research project with the Mass Spectrometry Group, Project Research Center for Fundamental Sciences, Graduate School of Science, Osaka University.
The tissue section specimen was provided by Awazu laboratory, Division of Sustainable Energy and Environmental Engineering, Graduate School of Engineering, Osaka University.

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