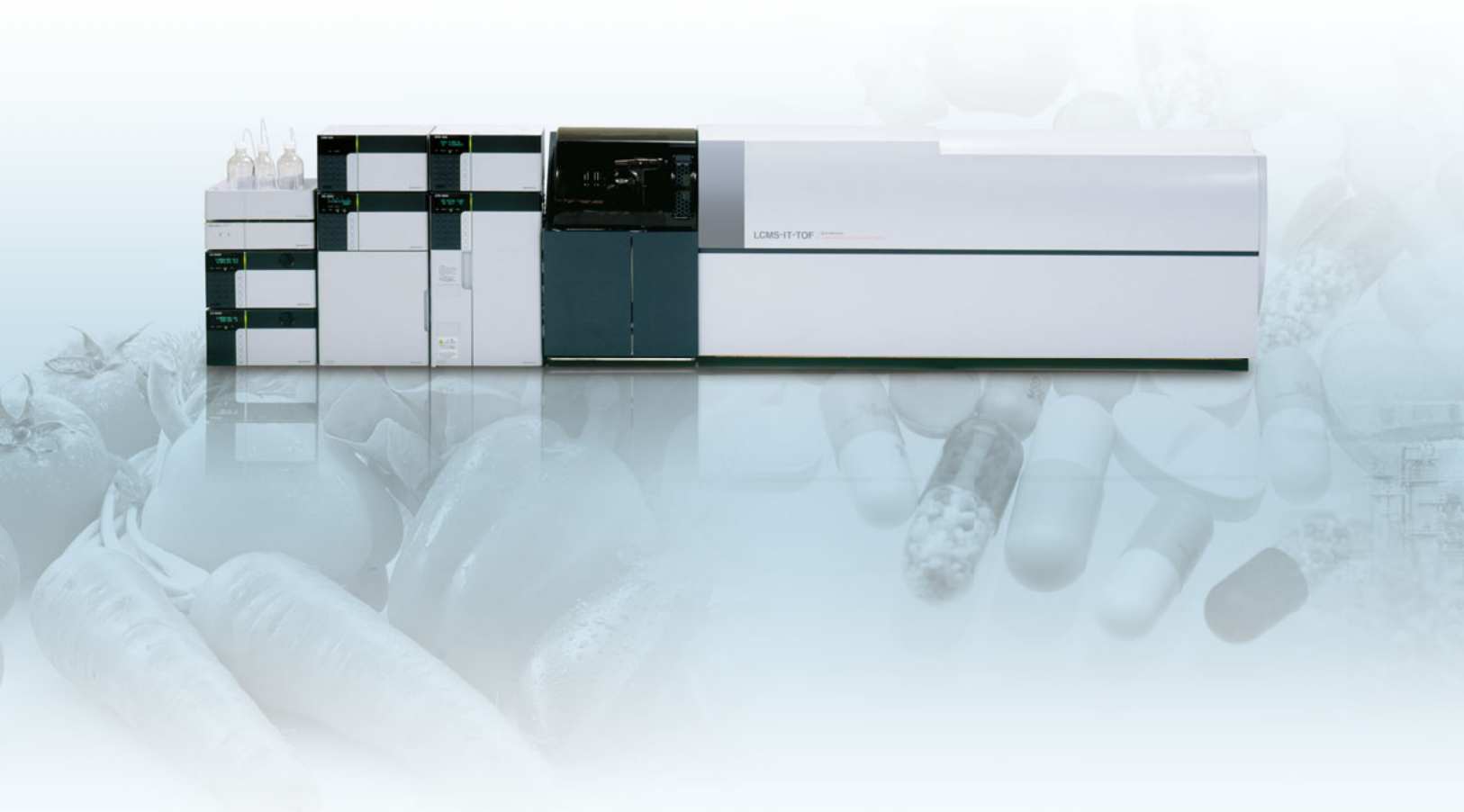
SHIMADZU
Excellence in Science

C146-E153A

Liquid Chromatograph Mass Spectrometer

LCMS-IT-TOF Solutions

LCMS-IT-TOF®



Providing New Solutions

LCMS-IT-TOF is a unique hybrid liquid chromatograph mass spectrometer that combines a high-performance liquid chromatograph, quadrupole ion trap mass spectrometer (QIT), and time-of-flight mass spectrometer (TOF) in a single integrated system. Combining the MS^n capability of the QIT unit with the precise, high-resolution mass measurement capability of the TOF unit enables high-accuracy MS^n mass measurements not possible with conventional LC/MS/MS systems.

The superior MS^n capability and high-resolution and high-accuracy mass measurement capability of LCMS-IT-TOF is utilized in a wide range of fields for various applications, from the structural analysis of trace impurities in drugs to the structural analysis of food ingredients. This catalog describes some analytical examples that take advantage of these outstanding capabilities.

Pharmaceuticals

- Raw materials for drugs
- Antibiotics and antimicrobials
- Herbal medicines and natural substances
- Veterinary drugs

Chemicals

- Plastics
- Solvents
- Paints
- Fibers and papers

Foods

- Food ingredients
- Additives
- Pesticide residues
- Fragrances

Life Sciences

- Biopolymers
- Sugars
- Proteins

Contents

P 06 Structural Analysis of Reserpine Degradation Products

P 08 Structural Analysis of Impurities in Erythromycin

P 10 Structural Analysis of Saponin

P 12 Structural Analysis of Unknown Polymer Additives

P 14 Structural Analysis of Additives in Alcoholic Beverages

P 16 Differential Analysis of Components in Green Tea

P 18 Structural Analysis of Flavonoids

P 20 Screening Analysis of Pesticides in Processed Foods

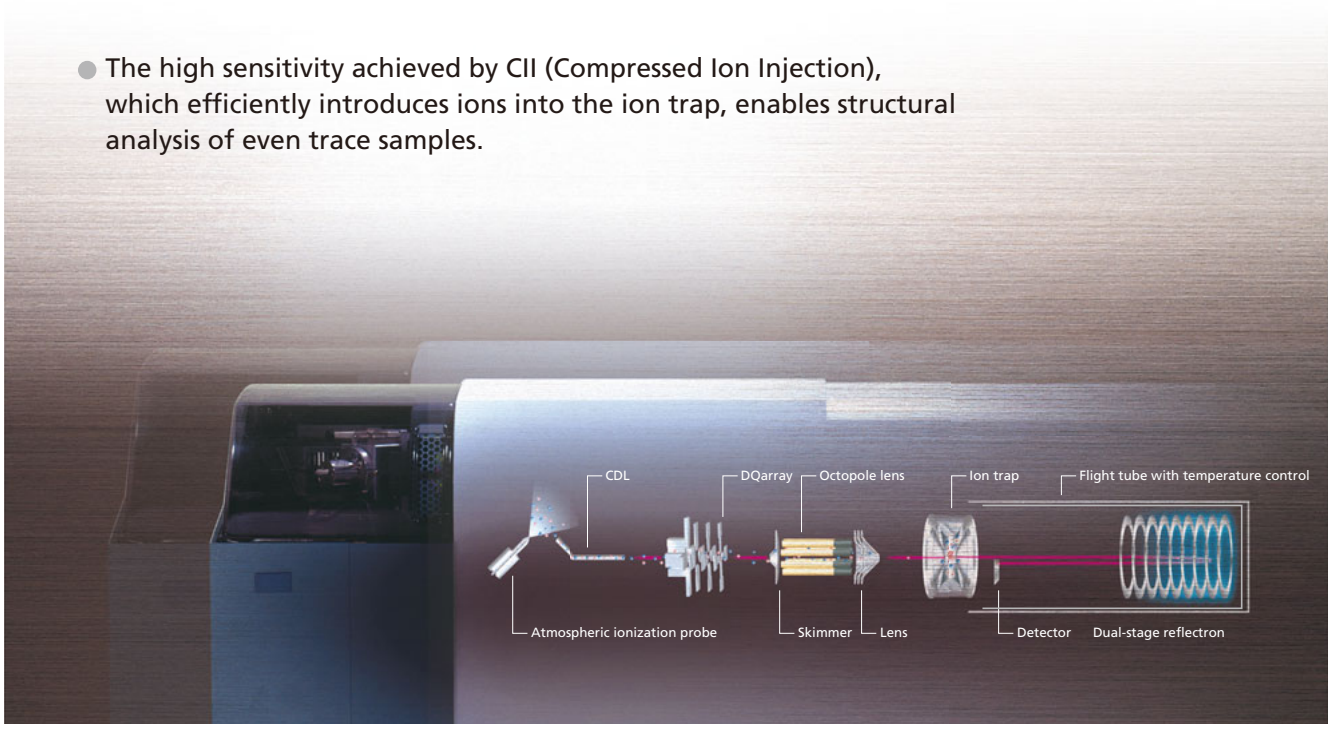
P 22 Identification of Molecular Species of Phospholipids

Advanced Hardware and User Friendly Software Provide New Solutions

- New technologies, Dual-Stage Reflection (DSR) and Ballistic Ion Extraction (BIE), enable obtaining high-resolution and high-accuracy MS^n data and accurately predicting molecular structures.

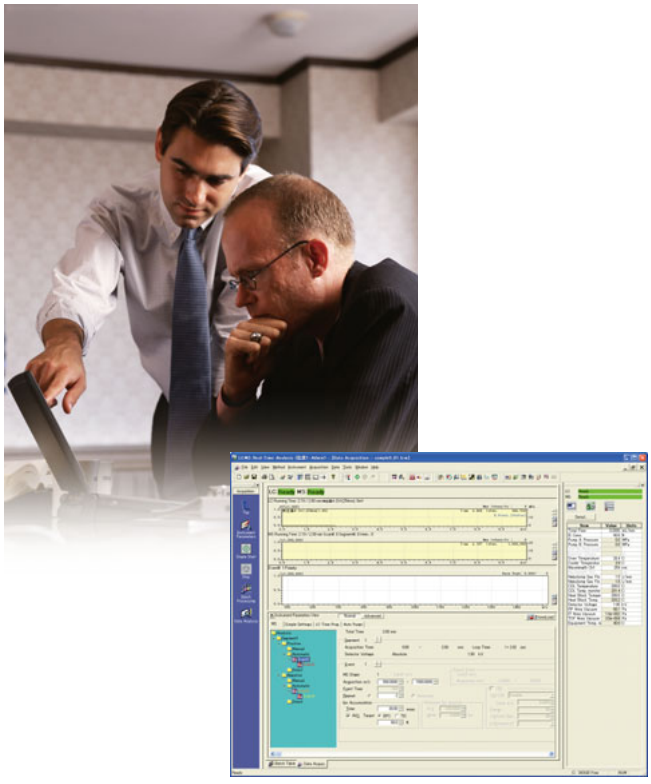
- The best-in-class high-speed mass spectra measurement performance and high-speed positive and negative ion polarity switching ensure highly reliable high-throughput structural analysis.

- The high sensitivity achieved by CII (Compressed Ion Injection), which efficiently introduces ions into the ion trap, enables structural analysis of even trace samples.



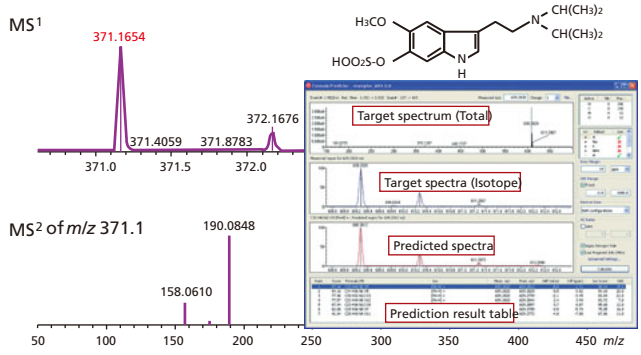
LCMSsolution Ver. 3.6

LCMSsolution Version 3.6 is dedicated software designed specifically for LCMS-IT-TOF. Software plays a very important role when using high-performance mass spectrometers. It not only allows adjusting the instrument, but it enables easily setting analytical conditions for a wide variety of measurement modes. The high-speed and high-accuracy data obtained with the LCMS-IT-TOF can be analyzed with minimal stress to provide the necessary information quickly and accelerate the overall workflow.



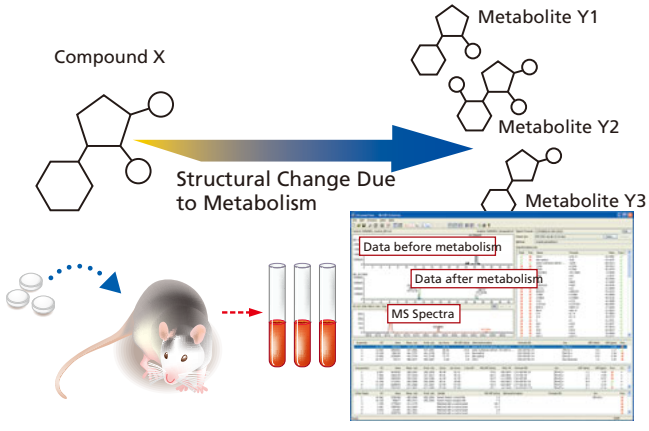
Composition Prediction Software (Formula Predictor)

The number of candidate ions can be efficiently reduced by first predicting the composition of smaller product ions from MS^n spectra measurement results, then using those results to predict precursor ions.



Metabolite Structural Analysis Software (MetID Solution)

Data is compared from samples before and after metabolism to search for expected and unknown metabolites.



Structural Analysis of Impurities in Drugs (Structural Analysis of Reserpine Degradation Products)

Introduction

Structural elucidation of impurities during the development of pharmaceutical products is very important. While NMR and other techniques can be used for this purpose, mass spectrometry is the primary means of conducting structural analysis. Therefore, the acquisition of highly accurate MS^n data and the high-speed analysis of large amounts of data are required.
This report describes the analysis of reserpine acid degradation products using the LCMS-IT-TOF, which obtains highly accurate MS^n mass data, which is analyzed using composition prediction, metabolite structural analysis, and other software to elucidate the composition and structure of the degradation products.

LCMS-IT-TOF Features

- The composition prediction software (Formula Predictor) not only allows obtaining highly accurate mass information, but also reduces the number of candidate impurities by using factors such as isotope patterns and MS^n information. Therefore, composition of impurities can be predicted with high reliability.
- The structural similarity search function of the metabolite structural analysis software (MetID Solution) enables rapid searching and analysis of impurities and their structures.

* Data was acquired with help from the Toray Research Center.

Results

Fig. 1 shows the total ion chromatogram (TIC) and the $[M+H]^+$ mass chromatograms of the 10 major compounds shown in the TIC.

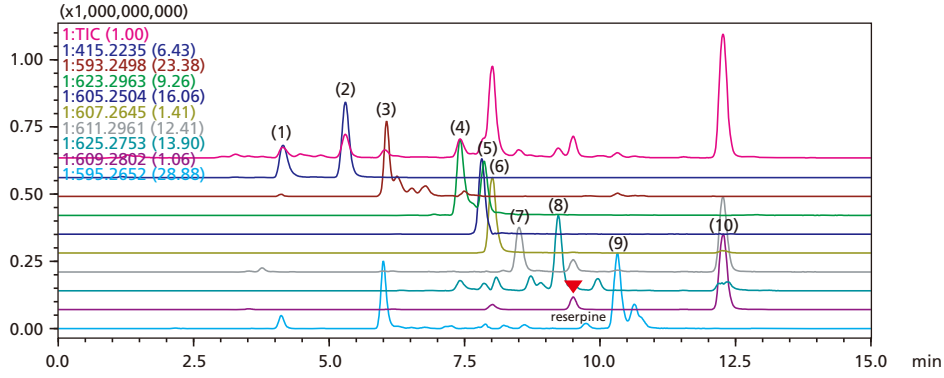


Fig. 1 Mass Chromatograms of the Acid Degradation Products of Reserpine

Fig. 2 shows the MS^2 mass spectrum for reserpine (precursor ion measured at *m/z* 609.2802). The reserpine cleavage points inferred from MS^2 product ion information and corresponding MS^3 information are indicated with arrows (Fig. 3). The data was analyzed using MetID Solution and the results are shown in Fig. 4. MetID Solution identified components with the same product ions or neutral loss as reserpine (Fig. 4a), which allowed the quick confirmation of the product ions or neutral losses in common with reserpine (Fig. 4b).

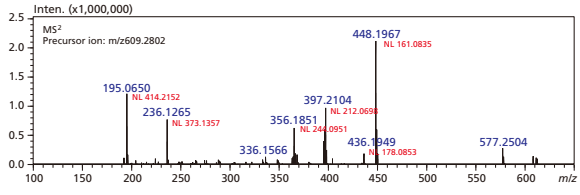


Fig. 2 MS^2 Mass Spectrum of Reserpine

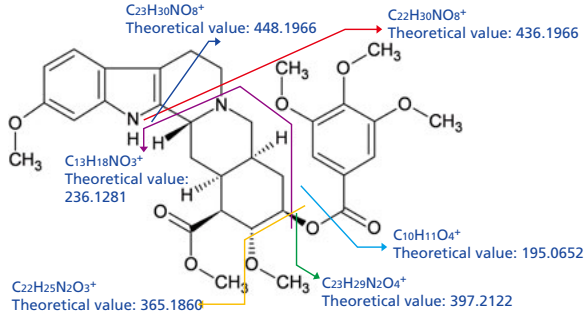


Fig. 3 Assignment of the Major Product Ions of Reserpine

As an example of analyzing the structure of reserpine degradation products, results from analyzing peak (3) (*m/z* 593) in Fig. 1 are indicated below.

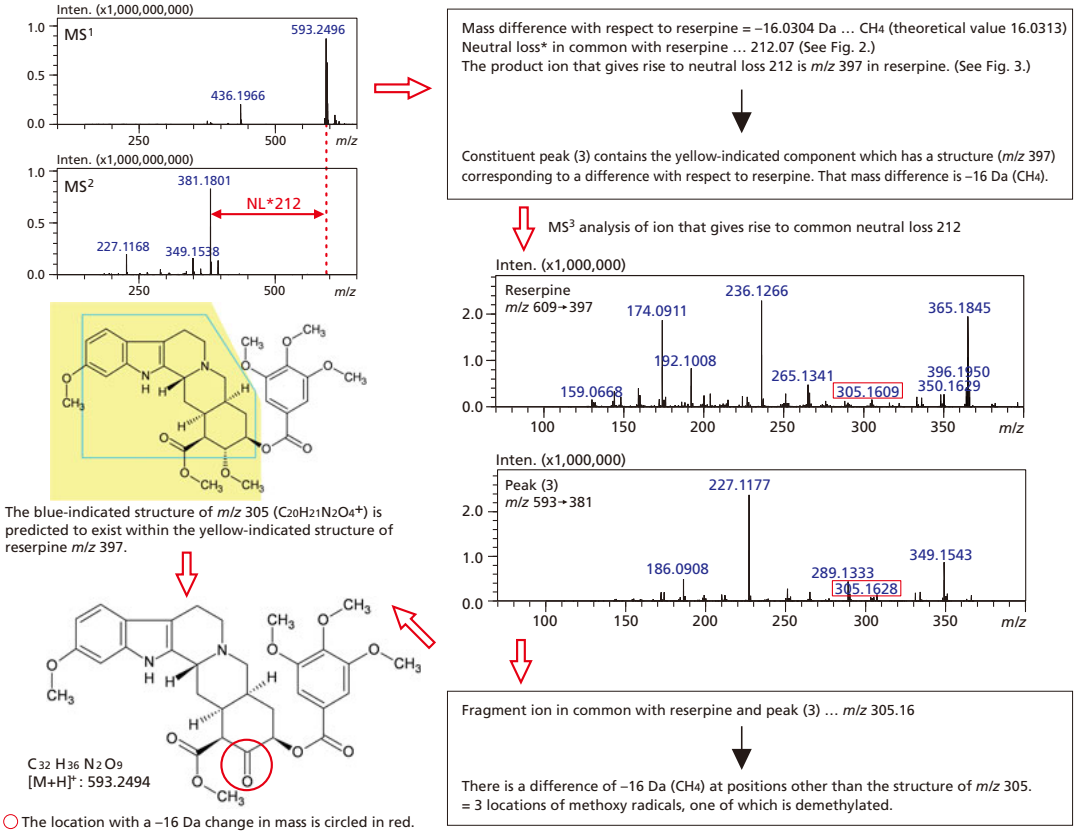


Fig. 4 Process of Predicting Structure of Peak (3) (*m/z* 593)

As shown, high-accuracy MS^n measurement is a useful tool for structural analysis of degradation products. Using the MS^3 analysis results for peak (3), the structure was predicted.
Regarding the other degradation products, peaks were analyzed using the same technique as for the peak (3), and the results are shown below. The composition formula corresponding to mass differences with respect to reserpine shown in Table 1 was predicted using the composition prediction software based on measured values.

Table 1 Summary of Analysis Results for Other Peaks

	Peak(1)	Peak(2)	Peak(3)	Peak(4)	Peak(5)	Peak(6)	Peak(7)	Peak(8)	Peak(9)	Peak(10)
Precursor ion (*m/z*)	415.2235	415.2238	593.2498	623.2963	605.2504	607.2645	611.2961	625.2753	595.2652	609.2797
Predicted composition	$C_{23}H_{30}N_2O_5$	$C_{23}H_{30}N_2O_5$	$C_{32}H_{36}N_2O_9$	$C_{34}H_{42}N_2O_9$	$C_{33}H_{36}N_2O_9$	$C_{33}H_{38}N_2O_9$	$C_{33}H_{42}N_2O_9$	$C_{33}H_{40}N_2O_9$	$C_{32}H_{38}N_2O_9$	$C_{33}H_{40}N_2O_9$
Variance with theoretical value (ppm)	1.93	2.65	0.67	0	1.65	-0.82	-0.33	-0.48	0.34	-1.64
Mass difference with respect to reserpine	-194.0567	-194.0564	-16.0304	+14.0161	-4.0298	-2.0157	+2.0159	+15.9951	+14.015	0
Composition indicated by difference	$C_{10}H_{10}O_4$	$C_{10}H_{10}O_4$	CH_4	CH_2	H_4	H_2	H_2	O	CH_2	–
Predicted existence position of difference	With in *m/z* 448	With in *m/z* 448	With in *m/z* 397 Out of *m/z* 305	With in *m/z* 365	With in *m/z* 397	With in *m/z* 305	With in *m/z* 236	With in *m/z* 305	With in *m/z* 397	–

Conclusions

- With its high-accuracy MS^n measurement, the LCMS-IT-TOF is a useful tool for structural analysis. Use of the LCMS-IT-TOF in conjunction with analysis support software, including Formula Predictor and
- MetID Solution, can greatly shorten the time required for structural analysis.

Technical Report Vol.20
(C146-E126)

Structural Analysis of Impurities in Drugs (Structural Analysis of Impurities in Erythromycin)

Introduction

Erythromycin is a macrolide antibiotic produced by a strain of bacteria known as *Saccaropolyspora erythraea*. The antibiotic is effective against many gram-positive and some gram-negative bacteria and is often used for people who display allergic reactions to penicillin. Structurally, this compound contains a 14-membered lactone ring (Area C) and two deoxy sugars, D-desoamine (Area A) and L-cladinose (Area B).

This report describes using composition prediction software (Formula Predictor) to identify the formulas and structures of impurities in an erythromycin A oxime sample. Discerning the chemical formula or structure of unknowns is a difficult task that can be partially alleviated by acquiring high mass accuracy data; however, data interpretation is tedious and time consuming. By using fragmentation spectra collected from the LCMS-IT-TOF along with enhanced composition prediction software, samples can be rapidly analyzed to identify chemical formulas and structures.

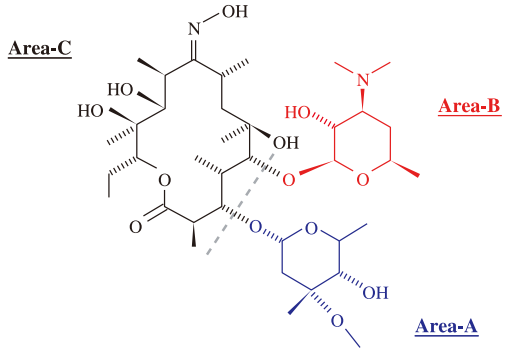


Fig. 1 Structure of Erythromycin A Oxime

LCMS-IT-TOF Features

- High-speed polarity switching and MS measurement functions ensure no important impurity molecules are overlooked.
- The composition prediction software (Formula Predictor) not only allows obtaining highly accurate mass information, but also reduces the number of candidate impurities by using factors such as isotope patterns and MS^n information. Therefore, composition of impurities can be predicted with high reliability.

Results

Fig. 2 shows the UV and mass chromatograms of erythromycin A oxime.

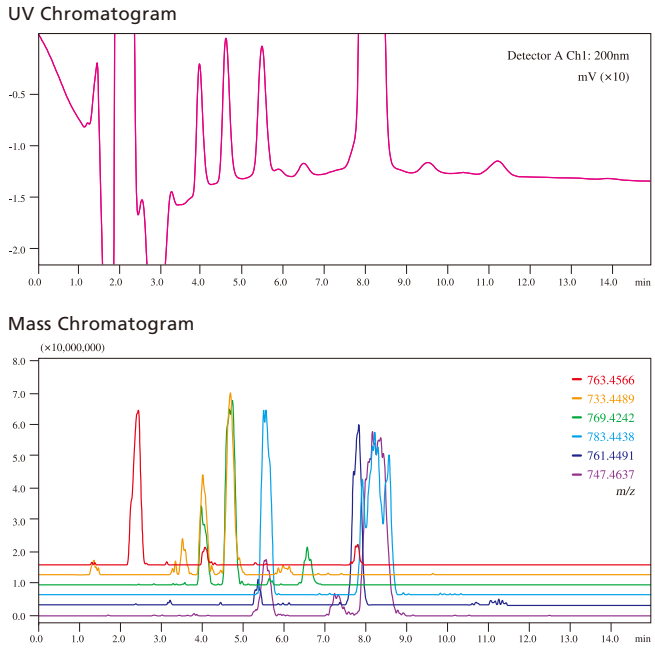


Fig. 2 UV and Mass Chromatograms of Erythromycin A Oxime Sample

Fig. 3 shows the mass spectra of impurity *m/z* 783.4421. As the *m/z* 396.2409 ions corresponding to Area C (Fig. 1) and the mass difference of 175.1165 indicating the loss of Area B (Fig. 1) in the MS^2 spectrum are the same as for erythromycin A oxime, this impurity is thought to be a 35.9841 Da change in Area A (Fig. 1) of erythromycin A oxime. Similarly, impurities *m/z* 733.4439 and 763.4581 are assumed to be the erythromycin A oxime-derived compounds since their mass patterns are alike.

Fig. 4 shows the mass spectra of impurity *m/z* 761.4375. The lack of a fragment ion at *m/z* 396.2392 in the MS^2 spectrum precludes the existence of Area C (Fig. 1). Also, as no losses of Area A (176.1049) or Area B (175.1208) in Fig. 1 are seen, the molecule is not thought to be an erythromycin A oxime-related compound.

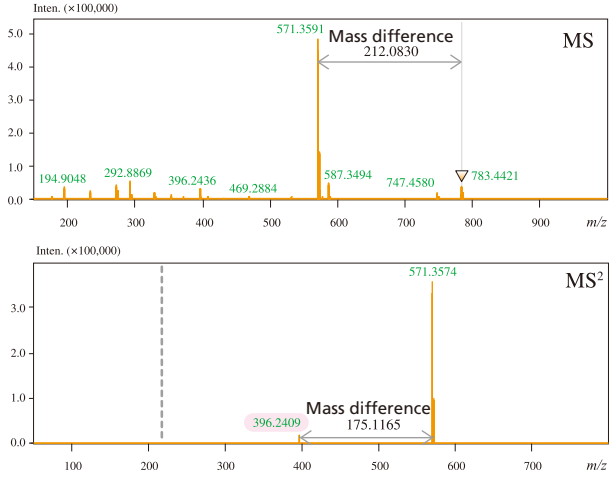


Fig. 3 Mass Spectra of Impurity (*m/z* 783.442)

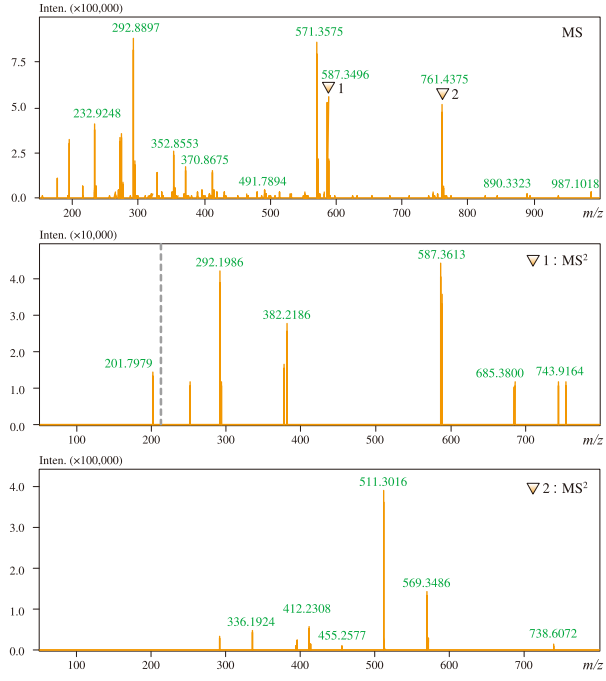


Fig. 4 Mass Spectra of Impurity (*m/z* 761.4375)

Conclusions

- Impurities *m/z* 733.4439, 763.4581 and 783.4421 are assumed to have structures similar to that of erythromycin A oxime since their mass patterns are alike. They are therefore believed to be derived from erythromycin A oxime.
- Since the MS2 spectrum patterns of the impurity at *m/z* 761.4375 are different from that of erythromycin A oxime, it is assumed that it was externally mixed into the sample.

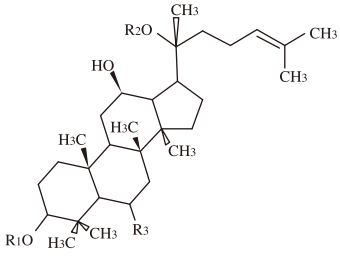
Technical Report Vol. 3
(C146-E104)

Structural Analysis of Saponin (Structural Analysis of Ginsenoside)

Introduction

Recently, the popularity of remedies consisting of natural products found in foods, roots and herbs has increased in both the domestic and global healthcare markets. These compounds, termed "nutraceuticals," refer to natural, biologically active chemical species that may be useful in disease prevention or have other additional medicinal properties. As a result of this renewed focus on nutraceuticals, efficient identification of active compounds in these products is a growing area of method development. This type of analysis requires a mass spectrometer that provides data with high resolution and high mass accuracy, combined with advanced structural prediction software.

This report describes analysis of the components in American ginseng using an LCMS-IT-TOF.



Ginsenoside	R_1	R_2	R_3
Rb1	-Glc^2-Glc	-Glc^6-Glc	H
Rb2	-Glc^2-Glc	-Glc^6-Ara(p)	H
Rc	-Glc^2-Glc	-Glc^6-Ara(f)	H
Rd	-Glc^2-Glc	-Glc	H
Re	-H	-Glc	-O-Glc^2-Rha
Rg1	-H	-Glc	-O-Glc

Fig. 1 Structure of Ginsenosides in Forms of Protopanaxadiol and Triol

LCMS-IT-TOF Features

- High-speed polarity switching and MS^n measurement functions ensure no important natural substances are overlooked.
- Composition prediction software (Formula Predictor) provides powerful support for structural analysis and for predicting the composition of natural substances.

Results

Fig. 2 shows the LC-MS chromatograms of American ginseng. Fractions A – F were collected with varying ratios of extraction solvent CH_2Cl_2:MeOH:H_2O, as indicated in the figure.

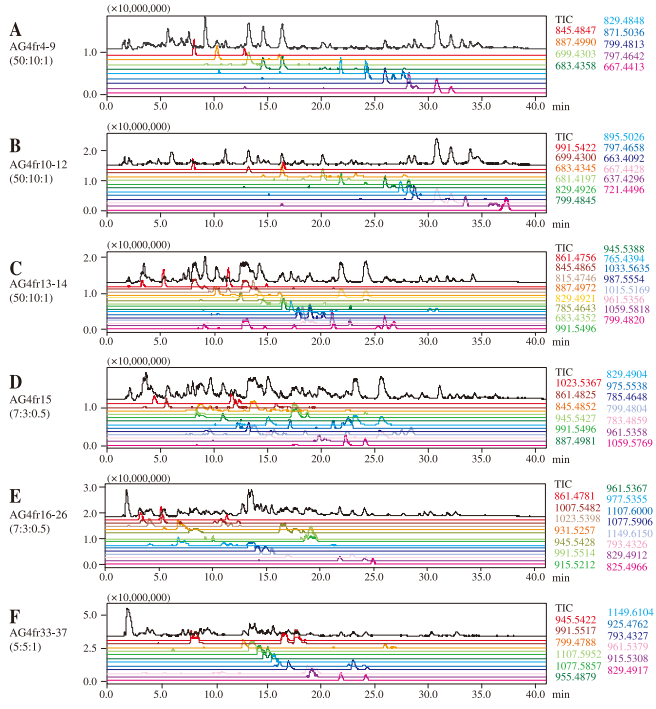


Fig. 2
LC-MS Chromatograms for Fractions of Extracted American Ginseng

Fig. 3 shows the mass spectra and expected dissociation pathway of ginsenoside Rb2 or Rc ($C_{53}H_{90}O_{22}$). It is shown that dissociation occurs first in the arabinose group and then in subsequent glucose groups.

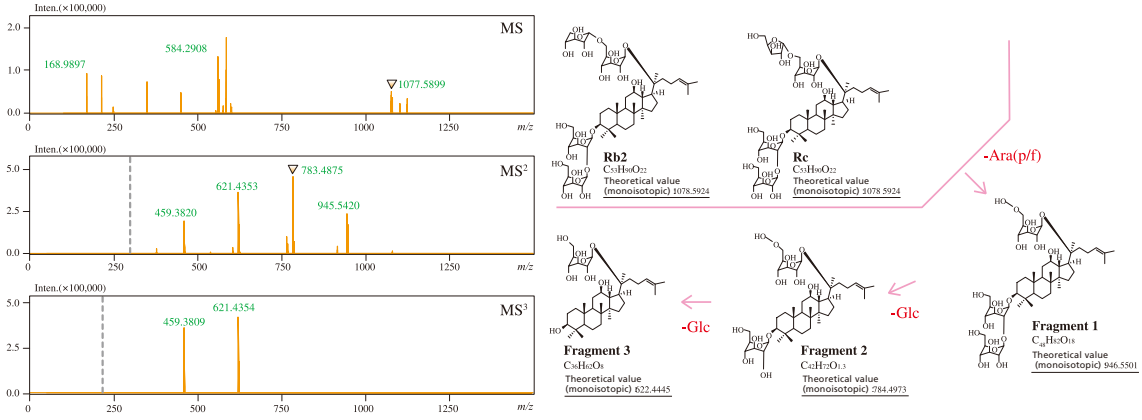


Fig. 3 Dissociation Pathway for Ginsenoside Rb2 or Rc ($C_{53}H_{90}O_{22}$)

The same method was used to analyze the ginsenoside components in American ginseng. Table 1 shows the mass accuracy data using the LCMS-IT-TOF for analyzing ginsenoside.

Table 1 Mass Accuracy Data for the Analysis of Ginsenosides Using the LCMS-IT-TOF

Compound Name	Formula	$[M-H]^-$ Theoretical Value (monoisotopic)	$[M-H]^-$ Measured Value (monoisotopic)	Error (ppm)
Rb1	$C_{54}H_{92}O_{23}$	1107.5951	1107.5979	2.5
Rb2 or Rc	$C_{53}H_{90}O_{22}$	1077.5845	1077.5906	5.6
Rd	$C_{48}H_{82}O_{18}$	945.5423	945.5420	0.3
Re	$C_{48}H_{82}O_{18}$	945.5423	945.5430	0.7
Rd/Re + formic acid	$C_{49}H_{84}O_{20}$	991.5478	991.5496	1.8
Ginsenoside Base	$C_{30}H_{52}O_4$	475.3787	475.3774	2.7*
Ginsenoside Base	$C_{30}H_{52}O_3$	459.3838	459.3825	2.8*
Rg1 + formic acid	$C_{43}H_{74}O_{16}$	845.4899	845.4868	3.7
F11	$C_{42}H_{72}O_{14}$	799.4844	799.4820	3.0
Ro	$C_{48}H_{76}O_{19}$	955.4903	955.4879	2.5
Rg3	$C_{42}H_{72}O_{13}$	783.4895	783.4859	4.6
Rg3 + formic acid	$C_{43}H_{74}O_{15}$	829.4949	829.4921	3.4
Rh1 + formic acid	$C_{37}H_{64}O_{11}$	683.4370	683.4352	2.6
Rh2 + formic acid	$C_{37}H_{64}O_{10}$	667.4421	667.4419	0.3
F1	$C_{36}H_{62}O_9$	637.4316	637.4296	3.1
Rs3	$C_{44}H_{74}O_{14}$	825.5000	825.4966	4.1
Notoginsenoside R1	$C_{47}H_{80}O_{18}$	931.5266	931.5250	1.7

* Mass accuracy of MS^3 spectra

Conclusions

- Ginsenosides from American ginseng were successfully separated and analyzed using the LCMS-IT-TOF. Adducts with formic acid and dimeric complexes were observed.
- Structural information and mass accuracy data can be obtained in a single experiment.
- Data with continuously high mass accuracy was obtained by simply auto-tuning before the experiment (for about 30 minutes).
- Fragmentation data successfully led to the correct assignment of ginsenosides with similar chemical formulae.
- The composition prediction software determined the composition of the unknown compound from the accurate mass data and fragmentation information obtained with the LCMS-IT-TOF.

Technical Report Vol. 4
(C146-E105)

Structural Analysis of Unknown Polymer Additives

Introduction

It would be very difficult to imagine what our lifestyle would be like without plastics. What is not so well-known is that synthetic polymers nearly always require polymer additives to achieve key performance properties.

For example, when plastics are exposed to heat and light, or when they come into contact with oxygen, they require the inclusion of heat stabilizers or antioxidants to keep their performance from degrading. There are thousands of common additives and mixtures used commercially, including ultraviolet light absorbing agents (UVA: Ultra Violet Absorbers) and stabilization agents (HALS: Hindered Amine Light Stabilizers). Even one polymer from a single manufacturer can contain different types of additives and blends of additives depending on the grade and intended use.For this reason, identifying the additires used in polymer materials is important as a means to adjust product characteristics with respect to competitors' products, and to improve products within a company's product line. This report introduces a unique method for identifying polymer additives, including unknown additives, using software capable of predicting their composition formula from highly accurate MS^n mass data and corresponding results.

LCMS-IT-TOF Features

- Reveals the structure of unknown additives using a combination of composition prediction software (Formula Predictor) and compound database information.
- Highly accurate MS^n mass measurements dramatically reduce the number of candidate composition formulas.

Results

Fig. 1 shows mass chromatograms of the supernatant obtained from ultrasonically processing commercial polymer beads in a THF/MeOH solution.

Peaks A, B, C and E were detected by ESI^+, peak D was detected by ESI^-.

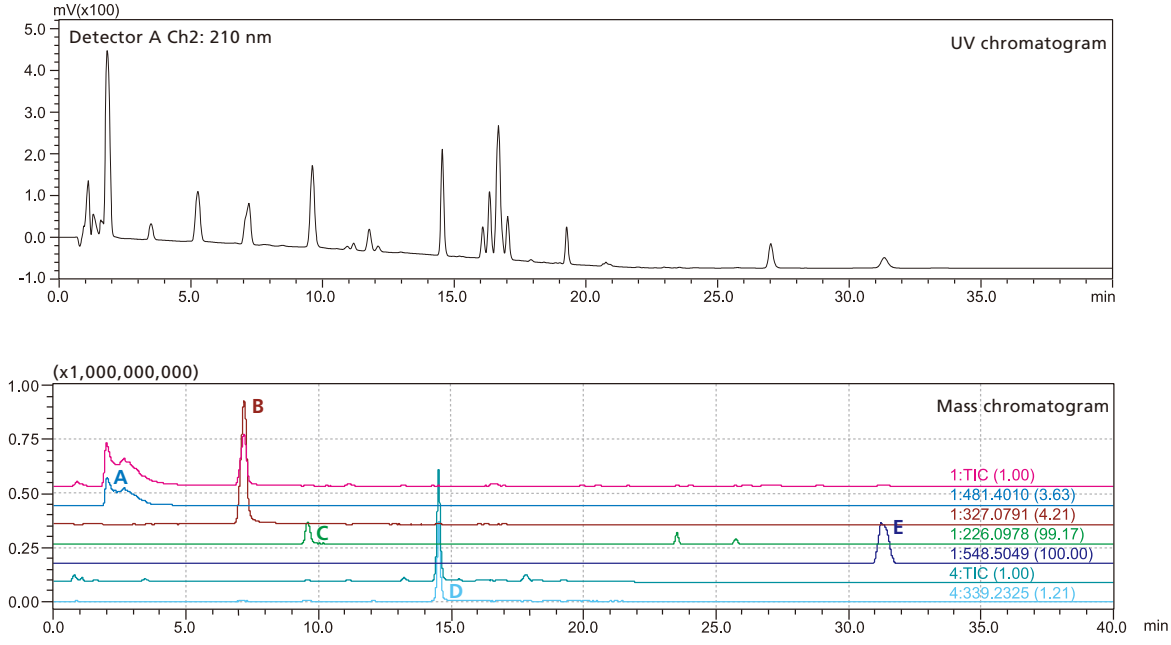


Fig. 1 UV Chromatogram and Mass Chromatogram

A Web search was performed for the formula ($C_{28}H_{52}N_2O_4$) for peak A, predicted using highly accurate MS, MS^2, and MS^3 mass data and Formula Predictor. Search results indicated that it could be decanedoic acid bis (2,2,6,6-tetramethyl-4-piperidyl) ester, commonly used as a stabilizer (Fig. 3). Attributing the ions detected in MS^3 measurements to the predicted compound indicated minimal error for each product ion and that they were consistent with the predicted structure (Fig. 4).

<Analysis of Peak A>

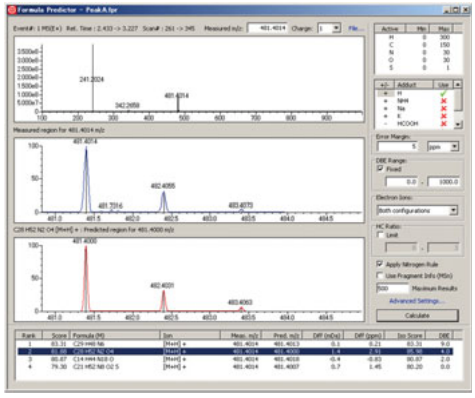


Fig. 2 Predicted Composition Results Using Formula Predictor

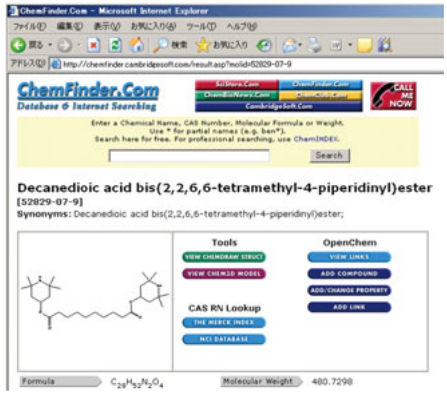


Fig. 3 Database Search Results Using ChemFinder

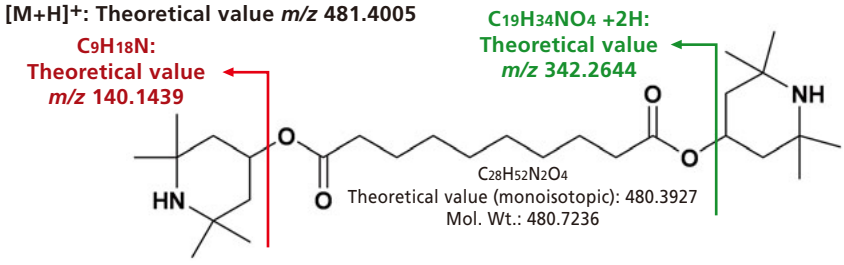


Fig. 4 Predicted Structure and MS^2 and MS^3 Spectra Assignments

<Identification Table>

Other peaks were identified by the technique used for peak A.
The predicted composition formula and mass accuracy for some of these compounds are summarized in the table below.

	Compound Name	MW	Formula	Theoretical Value $(M+H)^+$	Measured Value $(M+H)^+$	Error (ppm)	Theoretical Value $(M+H)^-$	Measured Value $(M+H)^-$	Error (ppm)
A	Decanedioic acid bis (2,2,6,6-tetramethyl-4-piperidyl) ester	480.3927	$C_{28}H_{52}N_2O_4$	481.4005	481.4014	1.87			
B	Triphenyl Phosphate	326.0708	$C_{18}H_{15}O_4P$	327.0786	327.0788	0.61			
C	Tinuvin P	225.0902	$C_{13}H_{11}N_3O$	226.0980	226.0981	0.44			
D	2,2-Bis (3-sec-butyl-4-hydroxyphenyl) propane	340.2402	$C_{23}H_{32}O_2$				339.2324	339.2322	-0.59
E	Irganox 1076	530.4699	$C_{35}H_{62}O_3$	548.5043	548.5067	4.38			

Conclusions

- Highly accurate mass information, in combination with Formula Predictor software, narrowed the composition formula candidates for peaks A, B, C, D, and E, detected in the polymer bead extract.
- Predicted structures for all the peaks (A, B, C, D, and E) were obtained by searching a compound database using the predicted composition as a keyword.
- The structure for all peaks (A, B, C, D, and E) was identified by comparing the accurate MS^n mass spectrum and predicted structure, revealing that all of them were additives contained in the polymer beads.
- This method allowed detecting the unknown additives in polymer beads.

Technical Report Vol. 8
(C146-E111)

Structural Analysis of Additives in Alcoholic Beverages (Structural Analysis of Sildenafil and Similar Compounds)

Introduction

The multivariate analysis (PLS: Partial Least Square) function, a feature in MetID Solution, is an effective approach for searching for and/or analyzing similar structures. In the structural prediction of impurities, the comparison of cleavage information with respect to the principal ingredient is important. MetID Solution only identifies candidate substances detected by LCMS-IT-TOF whose cleavage information is the same as that of the principal ingredient, enabling very smooth structural analysis. Here we present the measurement of an alcoholic beverage sample suspected of containing multiple compounds (sildenafils) similar to sildenafil, a principal ingredient in ED therapeutic drugs.

LCMS-IT-TOF Features

- Metabolite structural analysis software (MetID Solution) and composition prediction software (Formula Predictor) provide powerful support for structural analysis and for predicting the composition of similar structures.
- MetID Solution is especially useful when searching for and analyzing similar structures.
- Formula Predictor not only allows obtaining highly accurate mass information, but also reduces the number of candidate similar structures by using factors such as isotope patterns and MS^n information. Therefore, composition of similar structures can be predicted with high reliability.

Results

Fig. 1 shows the sildenafil mass spectra. The respective cleavage positions were predicted from the measurement values of the main fragmentation ions obtained from MS^2 analysis, as indicated by the arrow markings in the structural formula (Fig. 2). It is important to first predict the cleavage positions shown for the various fragmentation ions with respect to the compound used for comparison (sildenafil).

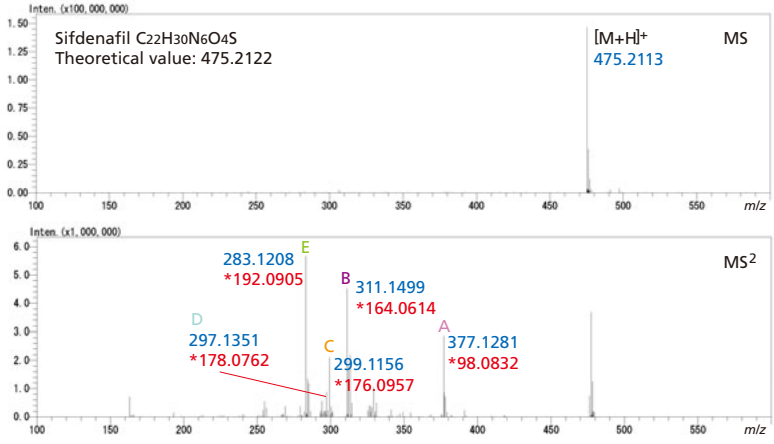


Fig. 1 Sildenafil Mass Spectra

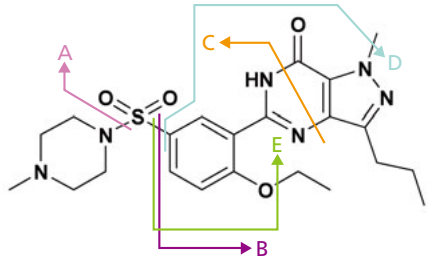
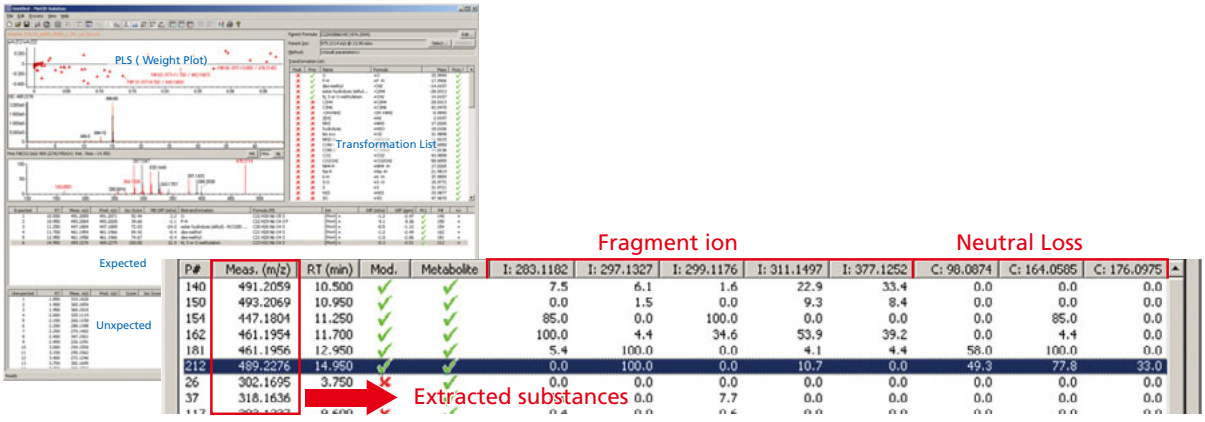


Fig. 2 Sildenafil Structural Formula and Predicted Cleavage Positions

Next, the data file is processed using MetID Solution. Fig. 3 shows the MetID Solution results window of the processed alcoholic beverage data file. In the PLS analysis, the substances with fragmentation ions or neutral loss in common with the principal ingredient take positive values along the X axis. Among the substances picked out as possible candidates, those in the Transformation List that have undergone change are listed as Expected, and substances other than those are listed as Unexpected. In addition, right-clicking on the main window displays the type of table shown at the lower right in Fig. 3. The items displayed in the columns in the table indicted as [I:xxx] are fragmentation ions observed in the principal ingredient (sildenafil), and the columns indicted as [C:xxx] indicate neutral loss. Substances assumed to have commonality with the principle ingredient can be quickly confirmed based on common elements of the cleavage information.



P#	Meas. (m/z)	RT (min)	Mod.	Metabolite	I: 283.1182	I: 297.1327	I: 299.1176	I: 311.1497	I: 377.1252	C: 98.0874	C: 164.0585	C: 176.0975
140	491.2059	10.500	✓	✓	7.5	6.1	1.6	22.9	33.4	0.0	0.0	0.0
150	493.2069	10.950	✓	✓	0.0	1.5	0.0	9.3	8.4	0.0	0.0	0.0
154	447.1804	11.250	✓	✓	85.0	0.0	100.0	0.0	0.0	0.0	85.0	0.0
162	461.1954	11.700	✓	✓	100.0	4.4	34.6	53.9	39.2	0.0	4.4	0.0
181	461.1956	12.950	✓	✓	5.4	100.0	0.0	4.1	4.4	58.0	100.0	0.0
212	489.2276	14.950	✓	✓	0.0	100.0	0.0	10.7	0.0	49.3	77.8	33.0
26	302.1695	3.750	✗	✓	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
37	318.1636				0.0	7.7	0.0	0.0	0.0	0.0	0.0	0.0

Fig. 3 PLS Analysis Results Using MetID Solution

As an example of the structural analysis of sildenafils, *m/z* 489 (P#212), highlighted in Fig. 3, was selected.

The measured value of the P#212 precursor ion is *m/z* 489.2276, and this value corresponds well with the values of the vardenafil (Fig. 4) and homosildenafil (Fig. 4) protonated molecules, known to be sildenafils (theoretical value of $C_{23}H_{32}N_6O_4S$: $[M+H]^+$ is 489.2279). Checking P#212 in the Fig. 3 table, the fragmentation ions (indicated as F) have common elements with the sildenafils *m/z* 297, 311, and the neutral losses (indicated as NL) have common elements with the sildenafils *m/z* 98, 164, 176, and 192, but among these, the existence of NL176 and NL192 becomes a critical factor in predicting the structure of P#212.

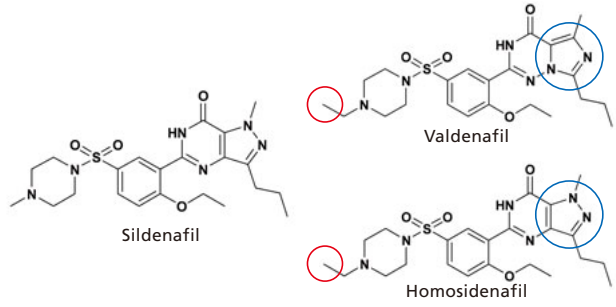


Fig. 4 Structural Formulas of Sildenafil, Vardenafil and Homosildenafil

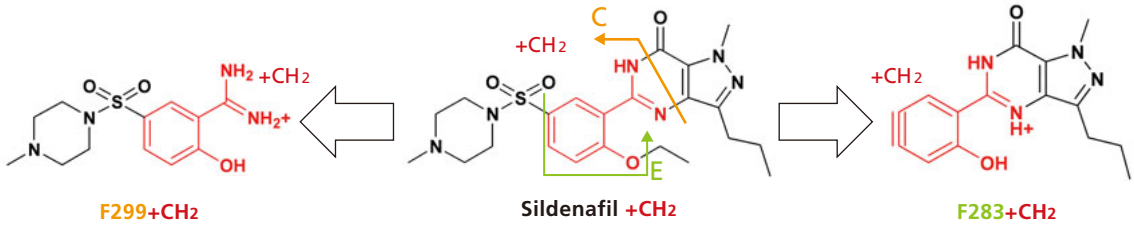


Fig. 5 Structure Prediction of P#212

NL 176 and NL 192 correlate with the neutral losses of F299 and F283, respectively (corresponding to Fig. 5 C and E). That is, it can be judged that the positions where CH_2 was added in P#212 are parts (displayed in red in structural formulas of Fig. 5) where F299 overlaps with F283. In other words, P#212 is seen to differ from vardenafil and homosildenafil in the positions of their methyl groups. To sum up, it is shown that P#212 is clearly neither vardenafil nor homosildenafil, and it is possible to narrow the positions where CH_2 was added.

Conclusions

- Using MetID Solution's PLS analysis function with MS^n information enabled the identification of components that have structures in common with primary components.
- Since information can be recalled quickly regarding which cleavage information is shared in common with primary components, it significantly reduces the amount of work required in subsequent structural analysis.
- MetID Solution software not only can be used to search for similar structures, but can also be used for a wide range of other functions, such as to analyze impurities.

Differential Analysis of Components in Green Tea Using Metabolomic Methods

Introduction

Metabolomics is a method of comprehensively analyzing metabolites. Since it can be used to analyze both known and unknown compounds, it is used in a wide range of applications, such as searching for diagnostic markers or analyzing pathogens in medical fields, searching for efficacy or toxicity markers in pharmaceuticals, and quality control and quality forecasting in food products. Many different techniques are used in metabolomics, but this report shows results from analyzing data from one commercial green tea beverage sample and two green tea leave extract samples. Using metabolomic techniques enables the analysis of samples with different properties as well as statistical analysis, which is used to identify candidate components corresponding to such differences in properties.

LCMS-IT-TOF Features

- The highly accurate MS, MS^2 and MS^3 mass measurement capability of LCMS-IT-TOF and multivariate analysis make differential analysis of trace components in foods simple.
- The highly accurate MS, MS^2, and MS^3 measurements make it easy to analyze the structure of trace substances.

Results

SIMCA-P (from Umetrics) was used for multivariate analysis. Score and loading plots from primary component analysis (PCA) are shown in Fig. 1. The fact that the score plot for each sample is separated in Fig. 1a confirms that measurement results differ for each sample. Furthermore, the fact that components such as EGC*1 and EGCG*2 appear toward the right of the loading plot in Fig. 1b indicates that large amounts of these components are present in the tea leaf extract solutions. In contrast, caffeine and theophylline are plotted toward the top, which indicates large amounts of these components are present in tea leaf A. The fact that CG is near the center indicates there is minimal difference in CG between the samples. In this way, primary component analysis results can be used to confirm differences in analytical results for each sample and clearly show the known compounds that indicate such differences.

In addition, there is an unknown compound (Compound A) near theophylline. Since this is plotted toward the top, it indicates a difference between tea leaf samples A and B. MS^n analysis was used to investigate this unknown compound (Compound A).

*1: Epigallocatechin
*2: Epigallocatechin gallate

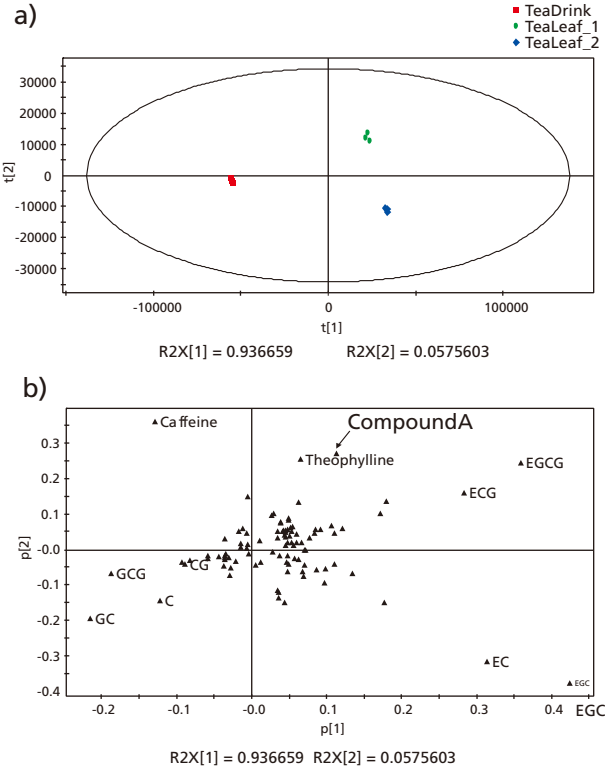


Fig. 1 Primary Component Analysis (PCA) Results - a) Score Plot and b) Loading Plot

MS^3 analysis results for Compound A are shown in Fig. 2 and the structure predicted using the composition prediction software is shown in Fig. 3. MS analysis alone resulted in four candidate composition formulas; MS^3 analysis reduced the number of candidates to two.

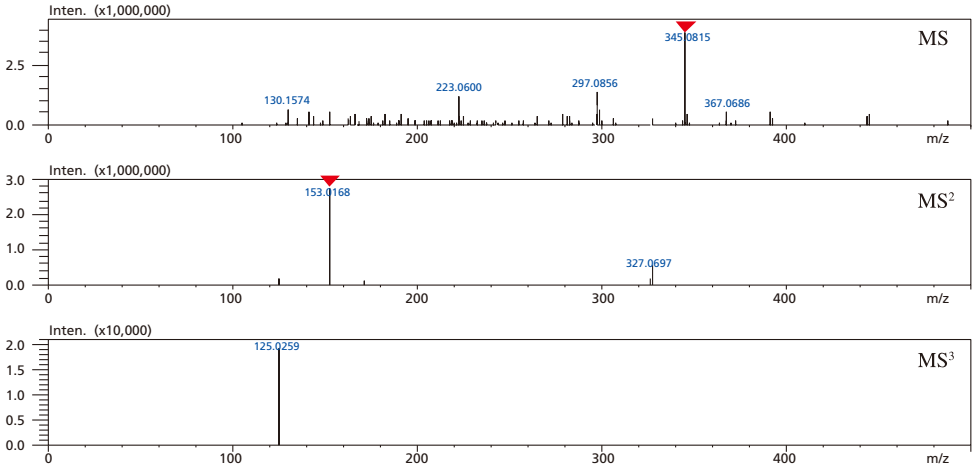


Fig. 2 MS, MS^2, and MS^3 Spectra of Compound A

In a search for the predicted formula in a database*3 published on the Web, it was determined the compound could be theogallin. An attempt to attribute MS^3 analysis results just obtained provided good results, with minimal difference between measured and theoretical values. Therefore, we were able to identify the compound was theogallin.

*3: In this case, CHEMnetBASE was used.

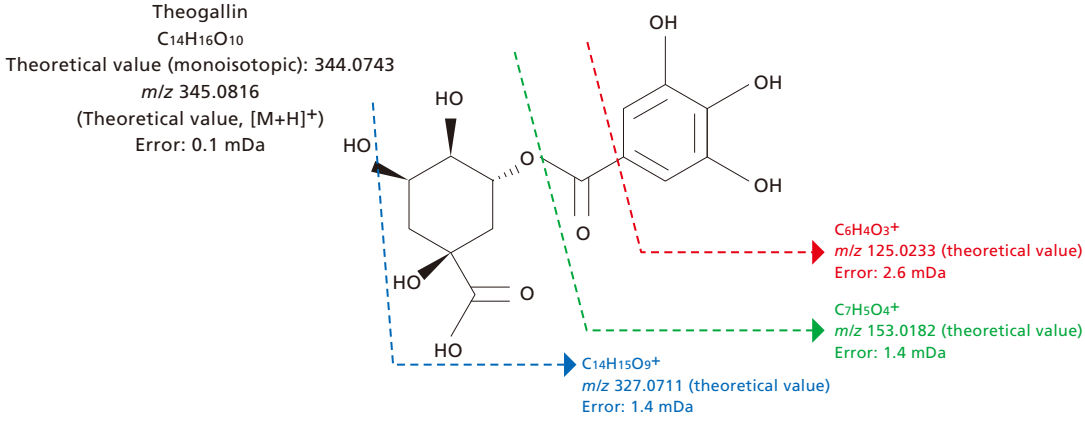


Fig. 3 Structural Formula of Theogallin and Attribution of MS^3 Analysis Results

Conclusions

- The highly accurate MS, MS^2, and MS^3 mass measurement data using the LCMS-IT-TOF combined with multivariate analysis techniques enabled the identification of differences between one commercial green tea beverage and two green tea leaf extract solutions.
- The analysis revealed the characteristics of the known compounds that differ between the commercial tea beverage and tea extract solutions. For unknown compounds, formula prediction results obtained from MS^3 analysis combined with a database search and other techniques enabled predicting their structure.
- Metabolomics is extremely useful for analyzing the differences between samples with different properties.

Acknowledgement

This research was made possible by the generous advice and cooperation from professor Eiichiro Fukusaki and associate professor Takeshi Bamba of the Division of Advanced Science and Biotechnology, Graduate School of Engineering, Osaka University.

Structural Analysis of Flavonoids

Introduction

Flavonoids, a kind of polyphenol, are present in fruits and vegetables, grains, seeds, nuts, wine and tea.
Determining the structure of a physiologically active flavonoid after discovering a minute quantity in a natural substance, and then separating and purifying it, takes a great deal of effort. However, using LCMS-IT-TOF to conduct accurate mass MS^n analysis, high-accuracy molecular weight information and structural information from fragment ions can be obtained without actually separating and purifying the substance.
In addition, unlike many existing TOF mass spectrometers, which achieve high mass accuracy using the internal standard method, the LCMS-IT-TOF attains long-lasting stability of mass accuracy using the external standard method, by utilizing unique technologies, including BIE (Ballistic Ion Extraction) and the internal temperature adjustment mechanism.
Here we introduce an analysis of mandarin orange methanol extract by LCMS-IT-TOF, and present the structural analysis results for the included flavonoids.

LCMS-IT-TOF Features

- Multiple iterations of MS^n data can be measured automatically in a single analysis using the automatic MS^n analysis feature and simply selecting precursor ions.
- Analyzing the mass spectra from MS^n measurements allows predicting the type, number, and position of glycosides, the structure of the aglycone portions, and other information.

Results

Fig. 1 shows the UV and MS chromatograms. Using the automatic MS/MS analysis feature, measurement up to MS^3 was conducted in one analysis while automatically selecting precursor ions. By conducting MS^n analysis, it was possible to predict the types, number and positions of the glycosides, as well as the structure of aglycon portions.

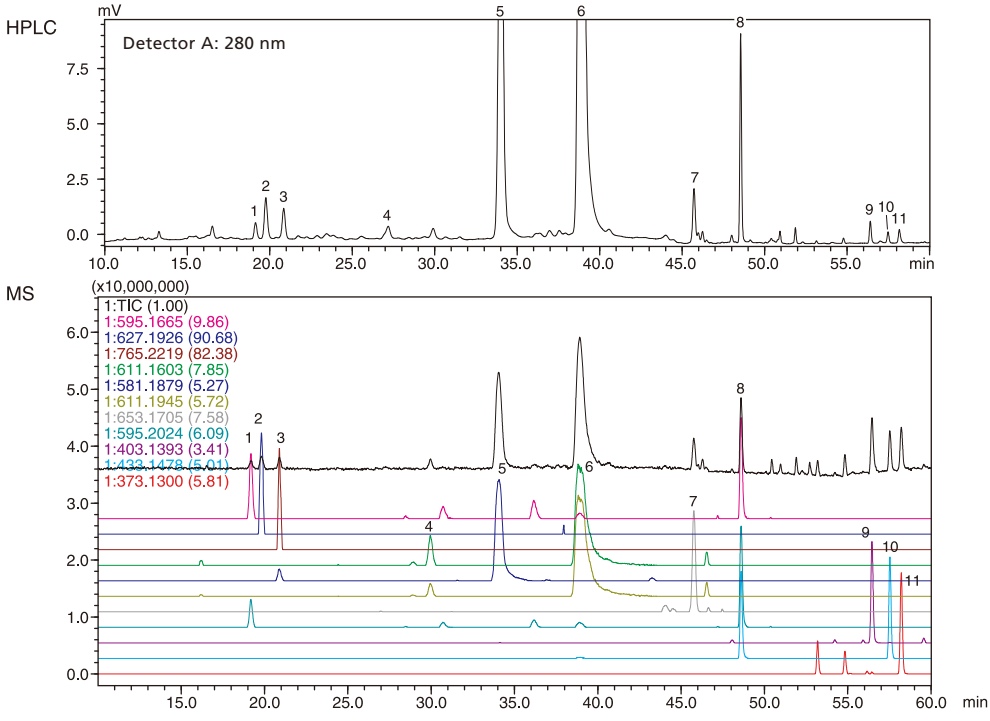


Fig.1 UV and MS Chromatograms of Mandarin Orange Methanol Extract

Fig. 2 shows the structure of the primary constituent, hesperidin (peak 6), and Fig. 3 shows the mass spectra of hesperidin.
The *m/z* 611.1945 indicated by the red ▼1 (Fig. 3) is the protonated hesperidin molecule $[M+H]^+$, with a theoretical value of m/z 611.1976, showing a mass accuracy of -5.1 ppm. Next, selecting this m/z 611 as the precursor ion, we collected an MS/MS spectrum.

Taking the differences between the precursor ion *m/z* 611 and fragmentation ions *m/z* 303 and *m/z* 449, respectively, yielded values of 308.1072 and 162.0532, and applying composition prediction to these values resulted in predictions of C12H20O9 and C6H10O5, respectively. These compositions correspond to the disaccharide and monosaccharide parts shown in Fig. 2. Next, we collected an MS/MS spectrum, selecting *m/z* 303 as the precursor ion. The predicted structures of *m/z* 145, *m/z* 153 and *m/z* 177 are shown in Fig. 2.

Using a similar technique, qualitative analysis was performed on the other peaks. Table 1 summarizes the qualitative results for each of the peaks, showing the theoretical and measured value of the ion related to the molecular weight associated with each peak. Good results were obtained, with the margin of error kept to within 5 ppm using the external standard method.

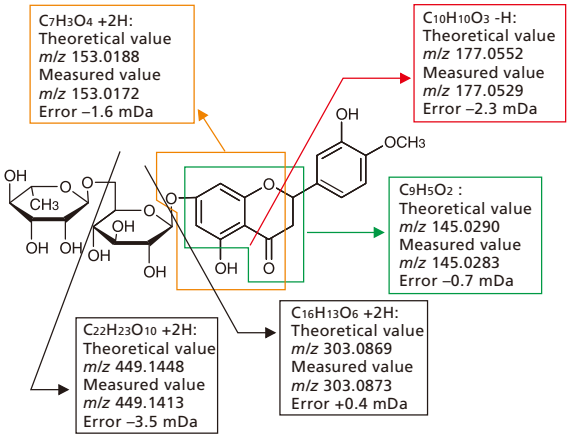


Fig. 2 Structure of Hesperidin

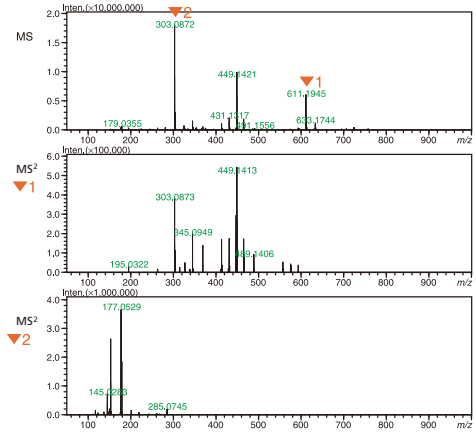


Fig. 3 Mass Spectra of Hesperidin

Table 1 Qualitative Results

	Retention Time (min)	Compound Name	Molecular Weight	Molecular Formula	Theoretical Value $[M+H]^+$ *m/z*	Measured Value $[M+H]^+$ *m/z*	Error (ppm)
#1	19.20		594.1585	$C_{27}H_{30}O_{15}$	595.1663	595.1665	+0.34
#2	19.82	3'-hydroxyhesperidin	626.1847	$C_{28}H_{34}O_{16}$	627.1925	627.1926	+0.16
#3	20.90	narirutin-4'-ß-D-Glu	742.2320	$C_{33}H_{42}O_{19}$	765.2218 $[M+Na]^+$	765.2219 $[M+Na]^+$	+0.13
#4	29.96	rutin	610.1534	$C_{27}H_{30}O_{16}$	611.1612	611.1603	-1.47
#5	34.06	narirutin	580.1792	$C_{27}H_{32}O_{14}$	581.1870	581.1879	+1.55
#6	38.93	hesperidin	610.1898	$C_{28}H_{34}O_{15}$	611.1976	611.1945	-5.07
#7	45.76		652.1640	$C_{29}H_{32}O_{17}$	653.1718	653.1705	-1.99
#8	48.60	5'-dehydroxyhesperidin	594.1949	$C_{28}H_{34}O_{14}$	595.2027	595.2024	-0.50
#9	56.46	nobiretin	402.1315	$C_{21}H_{22}O_8$	403.1393	403.1393	0
#10	57.53	3,5,6,7,8,3',4'- heptamethoxyflavone	432.1420	$C_{22}H_{24}O_9$	433.1499	433.1478	-4.85
#11	58.22	tangeretin	372.1209	$C_{20}H_{20}O_7$	373.1287	373.1300	+3.48

Conclusions

- The structure of each peak was identified by using the automatic MS^2 analysis feature and Formula Predictor composition prediction software.
- The external standard method kept the errors between theoretical and measured values for ions related to molecular weights for each peak within about 5 ppm.

Application News No.C51A
(LAAN-A-LM-E009)

Screening Analysis of Pesticides in Processed Foods

Introduction

The positive list system that prohibited sales of food containing pesticides, feed additives, and veterinary pharmaceutical products in greater than specified quantities was implemented on May 29, 2006 in Japan. Along with this, residue concentration criteria were established for about 800 pesticides. Businesses involved in the food market are requested to collect information regarding pesticides in food materials in the production stage and enforce stricter control of the qualities of these materials. Moreover, since residual pesticides in imported vegetables and processing foods are becoming a public concern, there is a demand for an easier method of quickly analyzing many pesticides at the site of import and export.

This report introduces an example of analysis of processed food extract spiked with the organophosphorus pesticides methamidophos and dichlorvos, etc. using the LCMS-IT-TOF according to the method indicated in the official notification of the Ministry of Health, Labour and Welfare dated March 7, 2008.

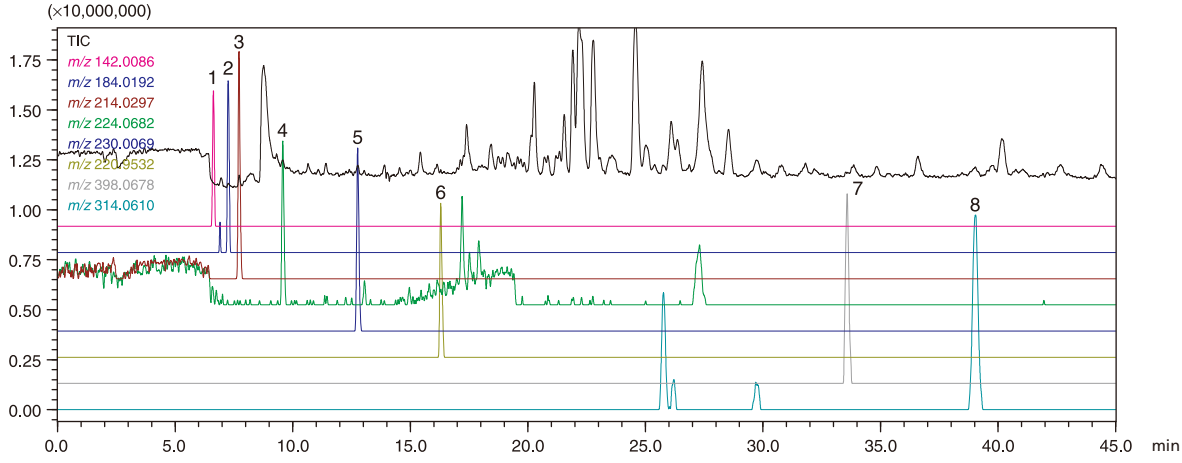
LCMS-IT-TOF Features

- The LCMS-IT-TOF is able to perform scan measurements down to the g/L level by switching between positive and negative modes every 100 milliseconds.
- Unknown pesticides can be qualitatively analyzed from the highly accurate MS^n mass spectra.

* The standard solution and processed food extract were provided by Dr. Mikiya Kitagawa of the Osaka Prefectural Public Health Laboratory.

Results

Fig. 1 shows the mass chromatograms of organophosphorus pesticides added to frozen dumpling extract at a concentration corresponding to 100 g/L.



Peak No.	Compound Name	Formula	*m/z*, Theoretical Value	Peak No.	Compound Name	Formula	*m/z*, Theoretical Value
1	Methamidophos	$C_2H_8NO_2PS$	142.0086	5	Dimethoate	$C_5H_{12}NO_3PS_2$	230.0069
2	Acephate	$C_4H_{10}NO_3PS$	184.0192	6	DDVP / Dichlorvos	$C_4H_7Cl_2O_4P$	220.9532
3	Omethoate	$C_5H_{12}NO_4PS$	214.0297	7	Bensulide	$C_{14}H_{24}NO_4PS_3$	398.0678
4	Monochlotophos	$C_7H_{14}NO_5P$	224.0682	8	Isoxathion	$C_{13}H_{16}NO_4PS$	314.0610

Fig.1 Mass Chromatograms of Organophosphorus Pesticides in Methanol Extract of Frozen Dumpling (100 g/L)

Fig. 2 shows the mass spectra of organophosphorus pesticides added to frozen dumpling extract at a concentration corresponding to 100 g/L. Using the LCMS-IT-TOF, which provides stable mass accuracy over a wide mass range by the external standard method, all of the pesticides were detected with a measurement error of less than 2 mDa. Moreover, the retention times were also the same as those of the standard substances, demonstrating that pesticides can be reliably identified.

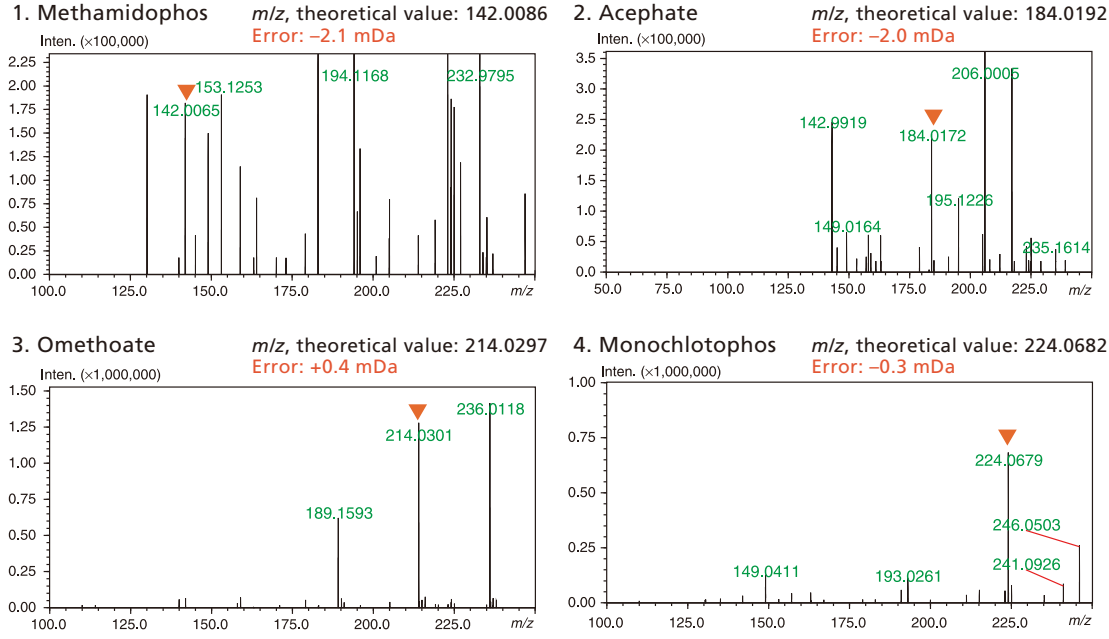


Fig. 2 Mass Spectra of Organophosphorus Pesticides in Methanol Extract of Frozen Dumpling

Fig. 3 shows the calibration curves of organophosphorus pesticides in the concentration ranges of 10 to 500 g/L. The calibration curve concentration range differs for the 2 pesticides due to the difference in ionization efficiency between the pesticides. Although the main purpose of the LCMS-IT-TOF is qualitative, quantitative analysis is also possible as evidenced by the excellent linearity and repeatability.

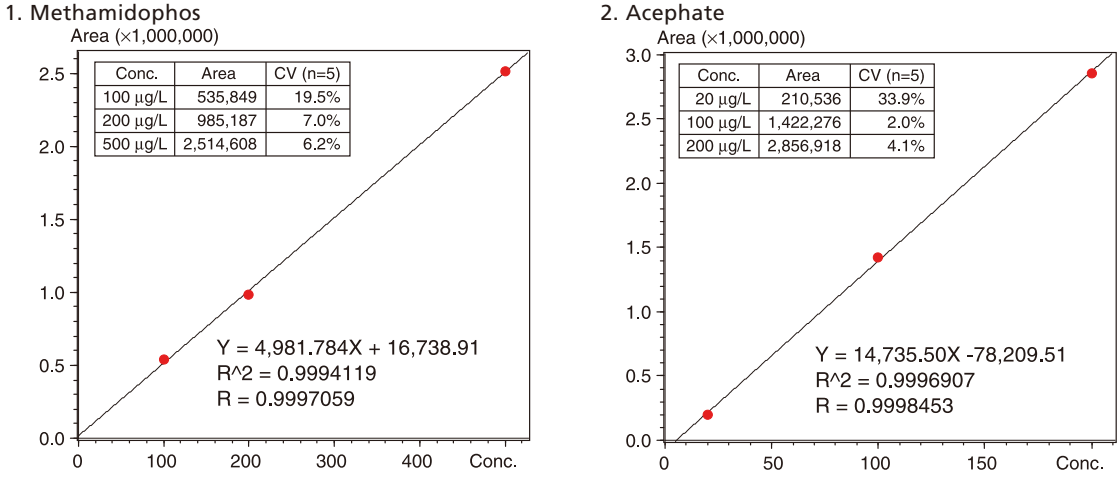


Fig. 3 Calibration Curves

Conclusions

- All pesticides were detected with a measurement error of less than about 2 mDa, which allowed positively identifying the pesticides.
- The LCMS-IT-TOF provided excellent linearity and repeatability even for quantitative analysis.

Application News No.C63
(LAAN-A-LM-E034)

Identification of Molecular Species of Phospholipids

Introduction

To elucidate the function of phospholipids, it is necessary to analyze their molecular species as well as their classes and subclasses. Electrospray ionization (ESI) MS^3 analysis is an effective technique for obtaining more detailed and accurate annotation of each molecular species. A system was established that encompassed analyzing molecular species of phospholipids with neutral loss survey of the head group-relating mass values, followed by MS^3 analyses, which involved selecting the resulting product ions as precursor ions (Fig. 1).Consequently, the 34 molecular species in phosphatidylcholine (PC) were identified without separating them by LC in advance. In addition, a method for analyzing phosphatidylethanolamine (PE) and phosphatidylserine (PS) in lipid mixtures was established.

LCMS-IT-TOF Features

- The NL survey (MS and MS^2) and subsequent MS^3 analysis enable high throughput lipidome analysis.
- The highly accurate MS, MS^2, and MS^3 measurements and NL survey provide powerful support for analyzing the molecular species of phospholipids.

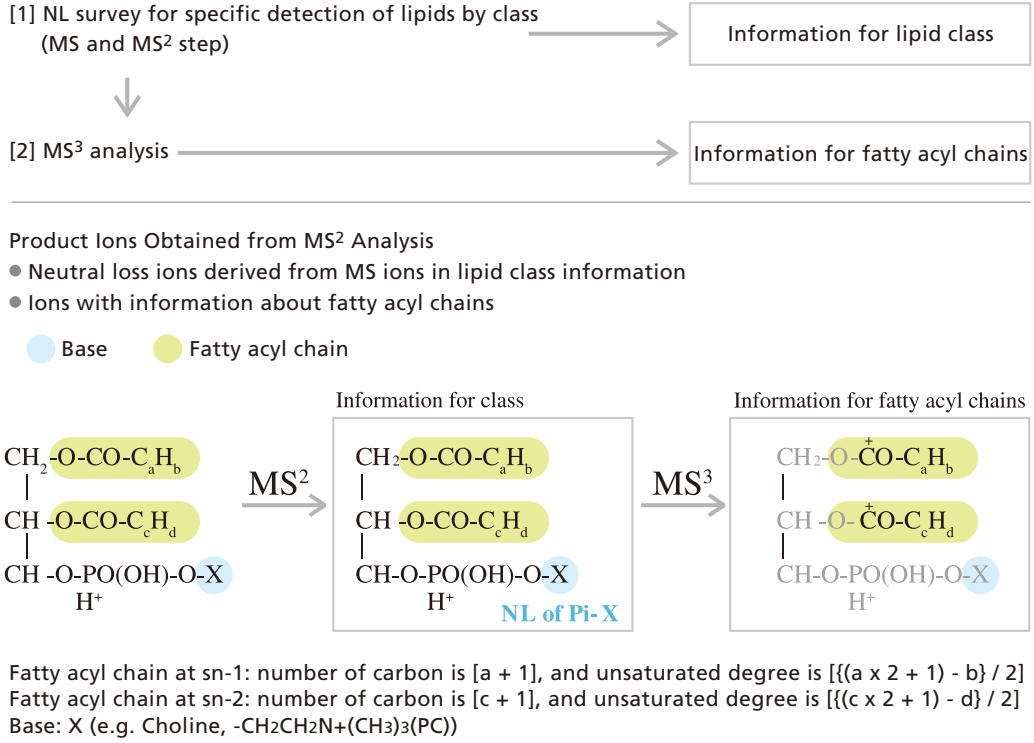


Fig. 1 System for Analyzing the Molecular Species of Phospholipids -Neutral Loss (NL) Survey and MS^3 Analysis-

Results

MS^2 analysis of $[M+H]^+$ ions from PE generates [M-phosphorylethanolamine (Pi-EthN)]$^+$ ions equivalent to [diglyceride OH]$^+$ ions as product ions in the positive mode (Fig. 2). MS^3 analysis with these [M-(Pi-EthN)]$^+$ ions selected as the second precursor ion generates [fatty acid (FA)-OH]$^+$ ions from the neutral loss of monoglyceride (MG) components and [MG-H]$^+$ ions from the neutral loss of FA-related components. This enables the efficient identification of fatty acyl chains in PE molecule species. MS^2 analysis of $[M-H]^-$ ions from PS generates [M-serine]$^-$ ions equivalent to [phosphatidate-H]$^-$ ions as product ions in the negative mode (Fig. 3). By selecting these [M-serine]$^-$ ions as the second precursor ion, fatty acyl chains of PS molecule species were identified from the corresponding [FA-H]$^-$ ions generated by MS^3.

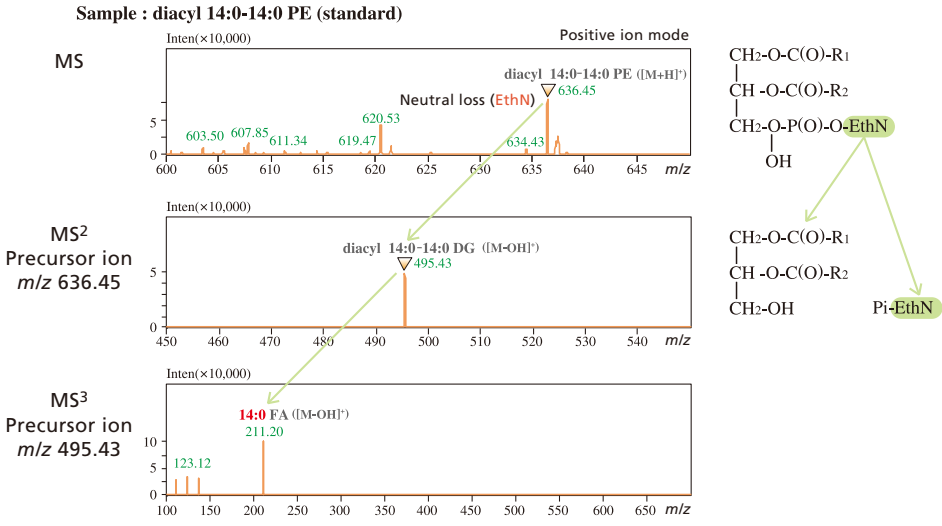


Fig. 2 PE Neutral Loss (NL) Survey and MS³ Analysis Results

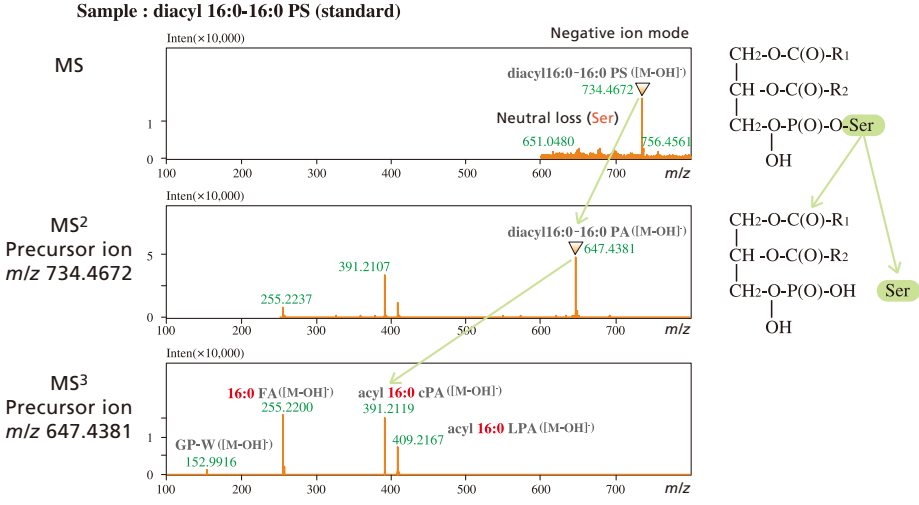


Fig. 3 PS Neutral Loss (NL) Survey and MS³ Analysis Results

Adding this new method to rapid MS³ analysis allowed identifying seven molecular species of PS. This NL survey combined with MS³ enabled identifying two phospholipid fatty acyl chains with high accuracy. The LCMS-IT-TOF provides additional highly accurate MS³ product ion mass information to NL survey information from MS² analysis, which was useful for identifying the two phospholipid fatty acyl chains with high reliability.

Conclusions

- By selecting 141 or 87 as the appropriate neutral loss scan condition, it was possible to identify PE and PS molecular species by separating them from other phospholipids.
- NL surveying (MS + MS²) and subsequent MS³ analysis is an extremely effective method of analyzing new organizational phospholipid classes.
- If IT-TOF is used, MS, MS², and MS³ mass accuracy is extremely high due to the external standard. The method of combining NL surveying with MS³ can be used to analyze the molecular species level of phospholipids.

Technical Report Vol. 2 (C146-E103)

Shimadzu Corporation
www.shimadzu.com/an/

Printed in Japan 3655-09201-30ANS