Applications note

High Performance Gas Chromatograph -
Time-of-Flight Mass Spectrometer

Solutions for Innovation

AccuTOF™ GC series

Environment, Food, Flavor, & Fragrance Applications Notebook

Edition February 2024

AI
AI
AccuTOF GC-Alpha

JEOL Ltd.

AccuTOF™ GC series

Environment, Food, Flavor, & Fragrance Applications Notebook

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MultiAnalyzer – Unknown Compounds Analysis System

New Gas Chromatograph Time-of-Flight Mass Spectrometer

JMS-T2000GC “AccuTOF^{TM} GC-Alpha”

Masaaki Ubukata MS Business Unit, JEOL Ltd.

The JMS-T2000GC AccuTOF™ GC-Alpha is the 6th generation JEOL GC-TOFMS and has an improved ion optics system to achieve ultra-high resolution. The AccuTOF™ GC-Alpha is an orthogonal-acceleration time-of-flight mass spectrometer(oaTOFMS) with dual stage reflectron. It employs an ideal ion optical system realizing both high ion transmission(=sensitivity) and ultra-high resolution. The dedicated qualitative software msFineAnalysis makes full use of the high-quality data obtained by the JMS-T2000GC AccuTOF™ GC-Alpha, thus providing a new approach to qualitative analysis for identification of unknown compounds. JEOL can offer real unknown compounds analysis solution with the powerful combination with JMS-T2000GC AccuTOF™ GC-Alpha and msFineAnalysis.

Introduction

Modern mass spectrometers (MS) are used for a variety of applications and scientific fields. Among these MS systems, the gas chromatograph-mass spectrometer (GC-MS) is suitable for both qualitative and quantitative analyses of volatile compounds and plays a crucial role in the analysis of materials, forensics, foods, environmental contaminants, etc. For qualitative analysis, GC-MS analysis typically involves collecting electron ionization (EI) mass spectra and then using these spectra for library database searches to identify each analyte. While this technique is effective for compounds found within these libraries, there are still many compounds that are not registered in the databases. As a result, additional tools are necessary to perform non-targeted analysis of unregistered components that can often appear during the analysis of materials and environmental samples. To address this situation, JEOL has continuously enhanced and improved upon our GC-MS products to include a variety of advanced capabilities, as shown in **Fig. 1**. The features improved upon include:

1) A Time-of-Flight Mass Spectrometer (TOFMS) that can acquire mass spectra with high resolution (HR) and high mass accuracy
2) Soft ionization techniques that are essential for determining the molecular weight and molecular formula of unknown substances
3) User-friendly software that automatically performs accurate mass analysis for the measured data

Our 6th generation GC-TOFMS, the JMS-T2000GC “AccuTOF^{TM} GC-Alpha,” was introduced into the market this year with significantly enhanced capabilities for higher mass resolution and higher mass accuracy. In this report, we will provide an overview of the AccuTOF^{TM} GC-Alpha instrumentation as well as its basic performance capabilities.

Overview of the Instrument

The new AccuTOF^{TM} GC-Alpha system is shown in **Fig. 2** and represents a significant design change from the previous models shown in Fig. 1. Specifically, the orthogonal acceleration (oa)-TOFMS ion trajectory was changed from the conventional “V”-like trajectory to a new “A”-like trajectory in order to extend the ion flight distance from 2 m to 4 m as shown in **Fig. 3**. Additionally, the oa-TOFMS was equipped with an improved ion acceleration system that spatially focuses the ion beam and an improved reflectron system that temporally focuses the ion beam. Because the system is a HRTOF-MS, the instrument is always operating in high resolution mode. Furthermore, by optimizing these components (along with the longer flight path), the AccuTOF^{TM} GC-Alpha represents a significant improvement in both mass resolving power and mass accuracy. The AccuTOF^{TM} GC-Alpha provides 6 times higher resolution than our 1st generation “AccuTOF^{TM} GC”.

Basic High-Performance System for Non-Targeted Analysis

The basic performance of the AccuTOF^{TM} GC-Alpha are as follows:

- Sensitivity: OFN 1 pg, S/N > 300
- Mass Resolving Power: > 30,000 (FWHM, *m/z* 614)
- Mass Accuracy: < 1 ppm (RMS, EI standard ion source)
- Mass Range: *m/z* 4 – 6,000
- Spectrum Recording Speed: Up to 50 spectra/sec
- Ionization Methods: EI, CI, PI, FI, DEI, DCI, and FD

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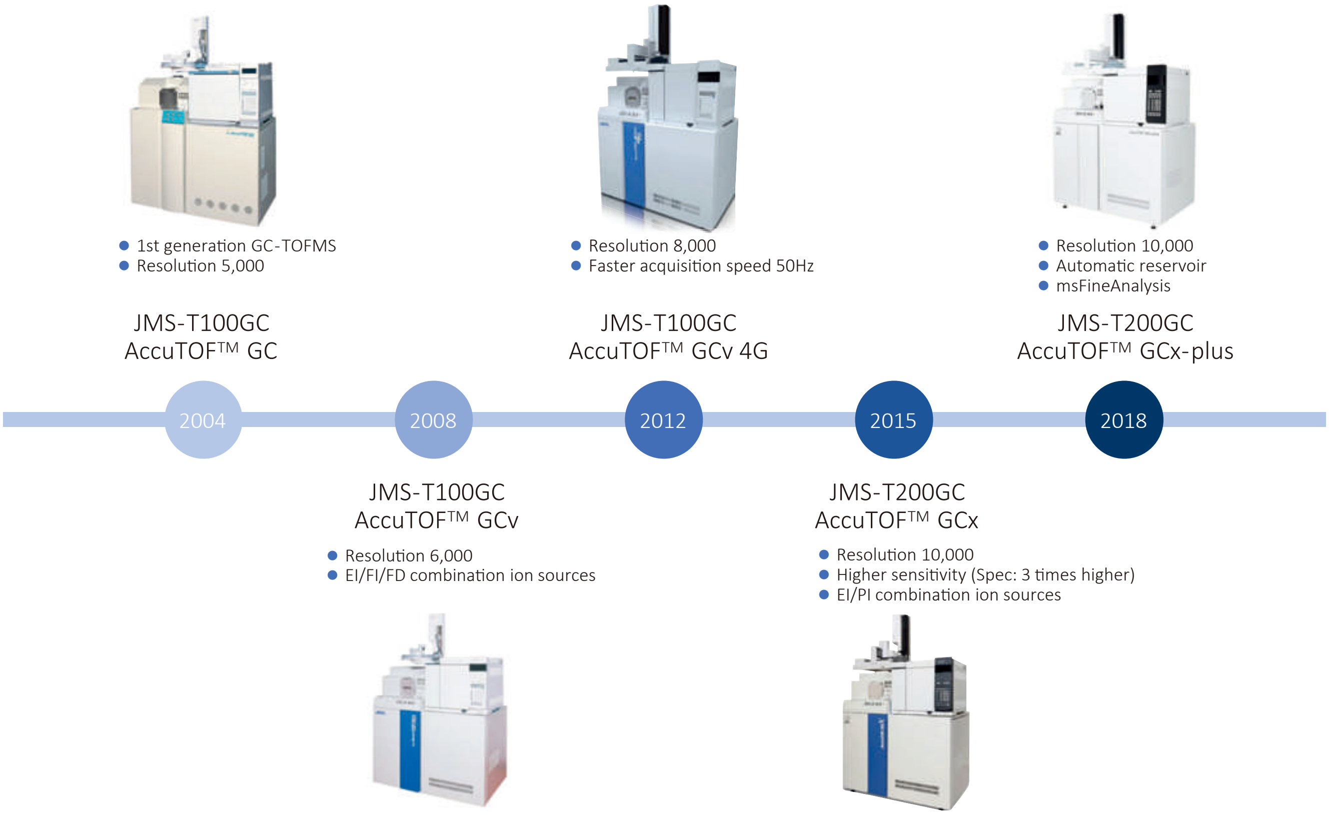


Fig. 1 History of JEOL GC-HR-TOFMS

● 1st generation GC-TOFMS
● Resolution 5,000
JMS-T100GC
AccuTOF™ GC
2004
2008
JMS-T100GC
AccuTOF™ GCv
● Resolution 6,000
● EI/FI/FD combination ion sources
● Resolution 8,000
● Faster acquisition speed 50Hz
JMS-T100GC
AccuTOF™ GCv 4G
2012
2015
JMS-T200GC
AccuTOF™ GCx
● Resolution 10,000
● Higher sensitivity (Spec: 3 times higher)
● EI/PI combination ion sources
● Resolution 10,000
● Automatic reservoir
● msFineAnalysis
JMS-T200GC
AccuTOF™ GCx-plus
2018

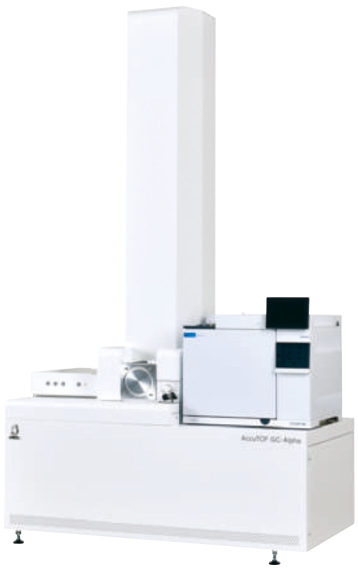


Fig. 2 JMS-T2000GC "AccuTOF™ GC-Alpha"

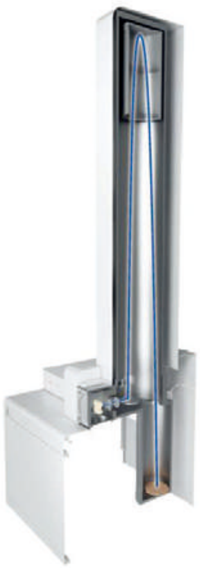


Fig. 3 Schematics of ion optical system

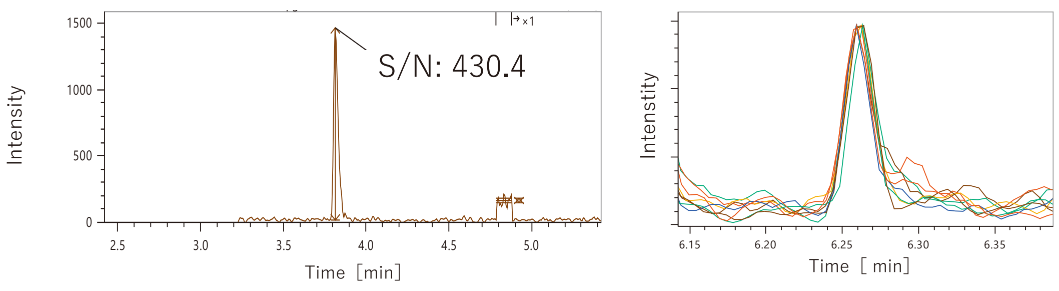
High Sensitivity

The new AccuTOF™ GC-Alpha has maintained the same sensitivity specifications as the previous generation system, despite the longer flight path and completely redesigned hardware. **Fig. 4**a shows the *m/z* 271.9867 extracted ion chromatogram (EIC) for 1 pg of octafluoronaphtalene (OFN) in which the GC-Alpha was able to easily achieve a S/N > 300. Fig. 4b shows the OFN EICs for the instrument detection limit (IDL) measurements in which 100 fg of OFN was injected 8-times. Additionally, Fig. 4b includes a table of peak areas, the calculated relative standard deviation (RSD) for these peak areas of 6.2%, and the calculated IDL of 18.7 fg. These results clearly demonstrate that the AccuTOF™ GC-Alpha provides both high sensitivity and high stability even for trace components analysis.

High Mass-Resolving Power

The AccuTOF™ GC-Alpha has a much higher mass-resolving power than the previous generations of the instrument. To demonstrate this new resolution capability, **Fig. 5**a shows the EI mass spectrum for PFTBA in which the *m/z* 614 peak was enlarged to clearly display the FWHM resolution of >30,000, which is the new resolution specification. To further highlight how this improved resolving power can enhance sample analysis, Fig. 5b shows the baseline separation of CO^+ (*m/z* 27.9949) and N_2^+ (*m/z* 28.0061) which was not possible with the previous generation instruments. These results clearly demonstrate that the high mass-resolution of the AccuTOF™ GC-Alpha can easily separate closely related ions (same nominal mass) and thus can be a powerful tool for a wide range of analyses and applications involving analytes with the same nominal mass but different elemental compositions.

Fig. 4 OFN 1 pg sensitivity and IDL for OFN 100 fg measurement

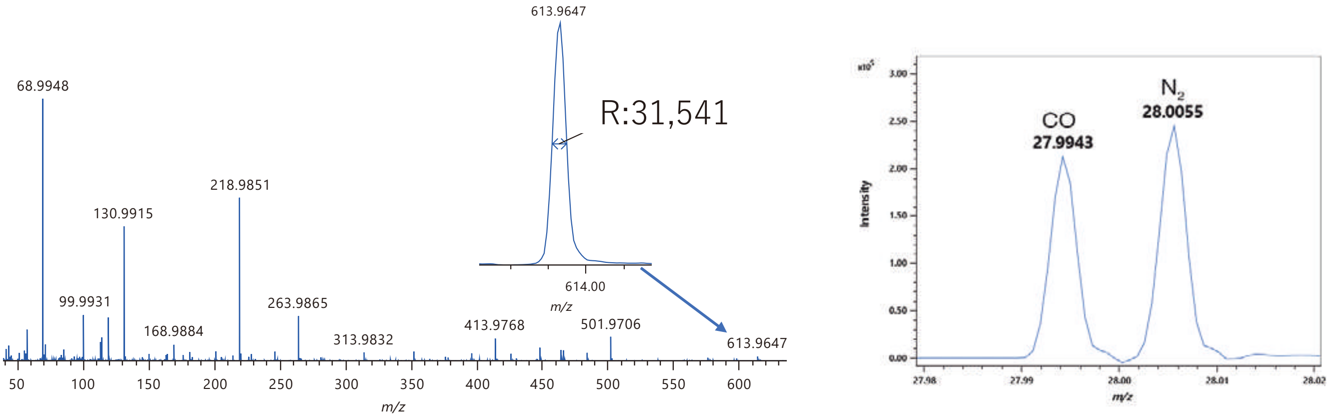


Extracted ion chromatogram of OFN 1 pg

Overlay of extracted ion chromatogram of OFN 100 fg (n=8) and its IDL

Inj. No.	Peak Area
1	1187
2	1239
3	1126
4	1210
5	1236
6	1088
7	1044
8	1123
RSD (%)	6.2
IDL (fg)	18.7

Fig. 5 EI mass spectrum of PFTBA and *m/z* 28 mass separation



EI mass spectrum of PFTBA

m/z 28 mass separation

High Mass-Accuracy

Along with higher mass resolving power, the AccuTOF™ GC-Alpha also has higher mass accuracy with a new specification of less than 1 ppm. An EI mass spectrum of methyl stearate along with the accurate mass analysis results for all observed ions are shown in **Fig. 6**. These results demonstrate that high mass accuracy is maintained over the entire mass range (from *m/z* 43 corresponding to the lowest mass fragment ion up to *m/z* 298 corresponding to the molecular ion), thus simplifying the determination of each compositional formula. Furthermore, the average mass error for these 10 ions was only 0.05 mDa / 0.45 ppm.

Also, worth noting here, the AccuTOF™ GC-Alpha produces high mass-accuracy mass spectra independent of the ionization method used for the measurements. This is important because the new system also offers all of the same ionization options that were available with the previous models - electron ionization (EI), chemical ionization (CI), photo ionization (PI), field ionization (FI) for GC-MS and desorption electron ionization (DEI), desorption chemical ionization (DCI), and field desorption (FD) methods for direct probe sample introduction. Therefore, the high mass accuracy of the AccuTOF™ GC-Alpha can be used with hard ionization (EI) for library searches / fragment ion formula determination and with soft ionization (CI, PI, FI) for molecular formula determination for the observed molecular ion / molecular ion adduct. This combination of mass accuracy and hard / soft ionization is a powerful tool for qualitative analysis of unknown compounds.

Powerful Capabilities for GC-MS Qualitative Analysis

- The AccuTOF™ GC-Alpha simultaneously achieves high sensitivity, high mass resolution and high mass accuracy.
- The AccuTOF™ GC-Alpha is suitable for not only target analysis but also non-targeted analysis of samples, even at trace levels.

Wide Mass Range

The AccuTOF™ GC-Alpha maintains the wide mass range (*m/z* 4-6000) of the previous generation systems. While GC-MS analysis is typically limited to analytes under *m/z* 1000, this increased mass range unlocks the analyst's ability to directly analyze samples like polymers and organometallics by using direct probe MS in which the sample is directly introduced into the ion source (not through the GC). The AccuTOF™ GC-Alpha accommodates two direct sample introduction probes (Direct Exposure Probe and Direct Insertion Probe) for direct EI measurement and direct CI measurement. Additionally, the optional FD probe allows the analyst to use a soft ionization direct probe method that is easy to use for the analysis of materials like polymers and higher molecular weight petrochemicals. Most commercial GC-MS instruments are dedicated only to GC-MS measurement, but the AccuTOF™ GC-Alpha can also be used as a direct MS instrument.

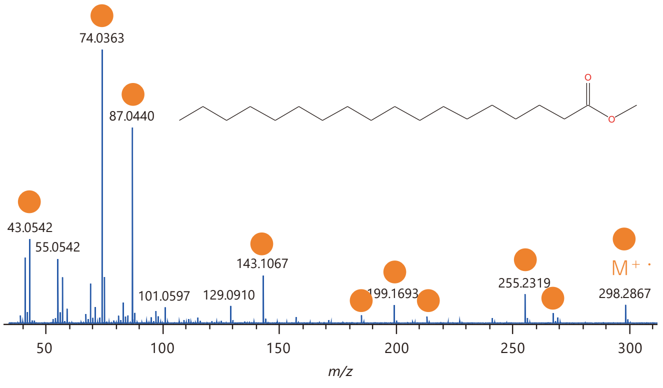
High-Speed Data Acquisition

The AccuTOF™ GC-Alpha maintains the high-speed data acquisition rate (up to 50 spectra/sec) of the previous generation systems as well. This capability is critical for doing Fast GC and GCxGC measurements which both require fast spectrum recording intervals. In particular, the AccuTOF™ GC-Alpha allows the user to bring together GCxGC separation, high resolution MS, high mass accuracy, EI, and soft ionization (CI, PI, FI) to analyze samples that are typically too complex for standard GC-MS separations.

Combined EI Method and Soft Ionization Methods – two combination ion sources –

As mentioned previously, library database searches are typically used for GC-MS qualitative analysis. However, for unknown compounds that are not registered in the MS databases, it can be difficult to identify these unknowns by using EI data alone. EI is a hard ionization method that typically produces many fragment ions as well as molecular ions. With that said, it is not uncommon for EI to produce minimal or no molecular ion signal in the mass spectrum. Consequently, the analyst can have difficulty deciding if

Fig. 6 EI mass spectrum of methyl stearate and the mass accuracy



EI mass spectrum of methyl stearate

Obs. *m/z*	Formula	Calc. *m/z*	Error [mDa]	Error [ppm]
43.0542	C3 H7	43.0542	-0.06	-1.42
74.0363	C3 H6 O2	74.0362	0.10	1.40
87.0440	C4 H7 O2	87.0441	-0.01	-0.16
143.1067	C8 H15 O2	143.1067	0.03	0.22
185.1537	C11 H21 O2	185.1536	0.05	0.26
199.1693	C12 H23 O2	199.1693	0.04	0.19
213.1850	C13 H25 O2	213.1849	0.08	0.39
255.2319	C16 H31 O2	255.2319	0.03	0.11
267.2683	C18 H35 O	267.2682	0.05	0.18
298.2867	C19 H38 O2	298.2866	0.06	0.20
Averaged mass error (Abs.)			0.05	0.45

there is a molecular ion present in the EI spectrum, thus complicating the data analysis. To overcome this problem, it can be very effective to use soft ionization methods to confirm the molecular ion / molecular adduct ion. The AccuTOF$^{\text{TM}}$ GC-Alpha has two optionally available combination ion sources that can be used to switch between EI and a soft ionization method (**Fig. 7**).

The EI/FI/FD combination ion source offers the combination of EI (hard ionization) and FI (soft ionization) for GC-MS measurements and FD (soft ionization) for direct probe MS measurements of heavier materials that do not go through the GC. Basically, this combination ion source allows the analyst to do GC/EI, GC/FI and FD measurements without breaking vacuum. All that is required to switch between ionization modes is the exchanging of the EI repeller probe with the FI/FD probe through a vacuum interlock that goes directly into the ion source. FI is the softest ionization method available (*Note) and will even produce molecular ions for saturated hydrocarbons.

The EI/PI combination ion source allows the AccuTOF$^{\text{TM}}$ GC-Alpha to be switched from GC/EI (hard ionization) to GC/PI (soft ionization) measurements without breaking vacuum. In this case, the ion source only requires switching between ON/OFF for the EI filament and ON/OFF for the PI ultraviolet-light lamp when switching between each ionization method. PI is particularly effective and sensitive for producing molecular ions for substances that can absorb ultraviolet light (e. g. aromatic compounds).

These unique combination ion sources are options only available with the AccuTOF$^{\text{TM}}$ GC-Alpha (see Fig. 1). They are extraordinarily powerful in that these sources allow the user to switch between GC/EI and GC/soft ionization without breaking vacuum while also capitalizing on the high mass resolution and high mass accuracy provided by the TOFMS. Thus, the AccuTOF$^{\text{TM}}$ GC-Alpha used with these combination ion sources simplifies the process of doing non-targeted, qualitative analysis of unknown compounds.

*Note: This case is applicable for ionization methods available with the AccuTOF$^{\text{TM}}$ GC-Alpha.

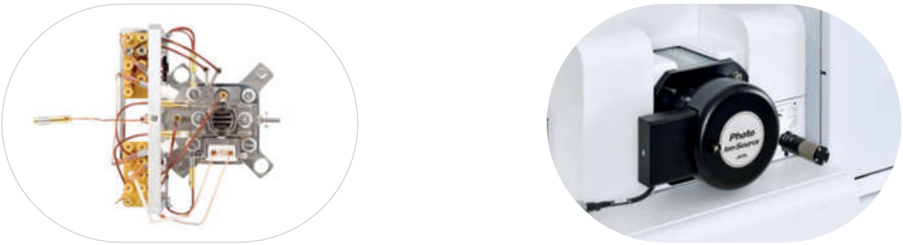
Automatic Qualitative Analysis Software "msFineAnalysis"

Originally released in 2018 as an option for the previous generation JMS-T200GC Series, the latest version of the "msFineAnalysis" software is now included with the basic AccuTOF$^{\text{TM}}$ GC-Alpha system. This software was designed to automatically identify compounds in a sample measured by GC-MS. More specifically, msFineAnalysis uses a new workflow that integrates GC/EI (hard ionization) high resolution data with GC/soft ionization (FI, PI, CI) high resolution data to automatically generate a color-coded qualitative analysis report for a measured sample. **Fig. 8** shows the integrated workflow used by the software in which five different analysis steps are automatically combined to produce fast, high-accuracy qualitative analysis results. The msFineAnalysis software is already widely acknowledged as a powerful tool for non-targeted analysis.

The latest version of msFineAnalysis (Version 3) streamlines the software operation and adds a new function in which two similar samples can be directly compared in order to identify sample differences. This feature can be particularly useful for comparing complex materials that have subtle differences. Identifying these differences can be critically important for addressing changes in material synthesis or manufacturing processes in which product quality is critically important.

As a starting point, the difference analysis function uses multiple GC/EI data to determine the components observed within the two samples (here referred to as A and B). Then, a t-test statistical analysis is done to extract the components that are different. Afterwards, a full integrated analysis (Fig. 8) is performed using the GC/EI data and GC/SI data. If a characteristic component in A or B is not registered in the library database, the

Fig. 7 Two combination ion sources



EI/FI/FD combination ion source

- EI and FI are switchable without breaking a vacuum.
- Replacement of the EI repeller probe and the FI emitter probe is needed.
- FD (direct MS) measurement is also possible.

EI/PI combination ion source

- EI and PI are switchable without breaking a vacuum.
- Replacement of the hardware is not required.
- PI ionization energy is about 10.3 eV.

integrated analysis will still determine an elemental composition for the unknown component. **Fig. 9** shows the difference analysis window which includes a volcano plot, classification section (A only, B only, A>B, A<B and A=B), intensity ratio (Log2(B/A), p value, etc. For each class, the components and analysis results are color coded to distinguish them from each other in order to enable a quick visual understanding of the different components.

As an example of difference analysis using msFineAnalysis Version 3, the evolved gas analysis results of two epoxy types of adhesives are shown in **Fig. 10**. A headspace GC-MS method was used for this analysis. The difference analysis parameters were as follows: Acquisition numbers were 5 for each GC/EI measurements, the significance level was 5%, and the threshold for intensity difference was set to 2. The upper section of Fig. 10 shows the TIC chromatograms (solid lines) as well as the detected peaks from chromatographic deconvolution. Each peak is color coded with blue indicating a characteristic component for adhesive A, red indicating a characteristic component for adhesive B, and yellow indicating a component that is observed in both A and B (no difference). The blue and red color scheme is also applied to the integrated analysis report in the lower section of the window, but white is used in this case to identify the components observed in both A and B (no difference). The difference analysis results for this example showed:

- Toluene was observed in both adhesives.
- Adhesive A had a high intensity peak for butanol that was not observed from adhesive B.
- Adhesive B had several aromatic compounds such as ethylbenzene and xylene.
- Adhesive B had cyclic siloxanes.

For conventional GC-MS difference analysis, if the extracted characteristic component is not registered in the library database, then it can be difficult to identify this unknown component. However, using msFineAnaysis Version 3 makes it possible to automatically identify these components using the integrated analysis workflow that is subsequently performed after the difference analysis. This innovative 2-sample comparison solutions can be broadly applied to not only the materials field but also to a variety of other research and application fields.

Conclusions

The new JMS-T2000GC “AccuTOF$^{\text{TM}}$ GC-Alpha” is a new high-resolution GC-TOFMS that represents a significant improvement over the previous generation instruments. The hardware was completely redesigned for improved resolving power, mass accuracy, sensitivity and stability. Along with the improved hardware of the AccuTOF$^{\text{TM}}$ GC-Alpha, the automatic qualitative analysis software “msFineAnaysis” was also upgraded to incorporate difference analysis between two-samples while also continuing to capitalize on the integrated analysis workflow that this software is known for. The combination of AccuTOF$^{\text{TM}}$ GC-Alpha and msFineAnalysis is a truly powerful solution for GC-MS qualitative analysis.

Fig. 8 msFineAnalysis: Integrated analysis workflow

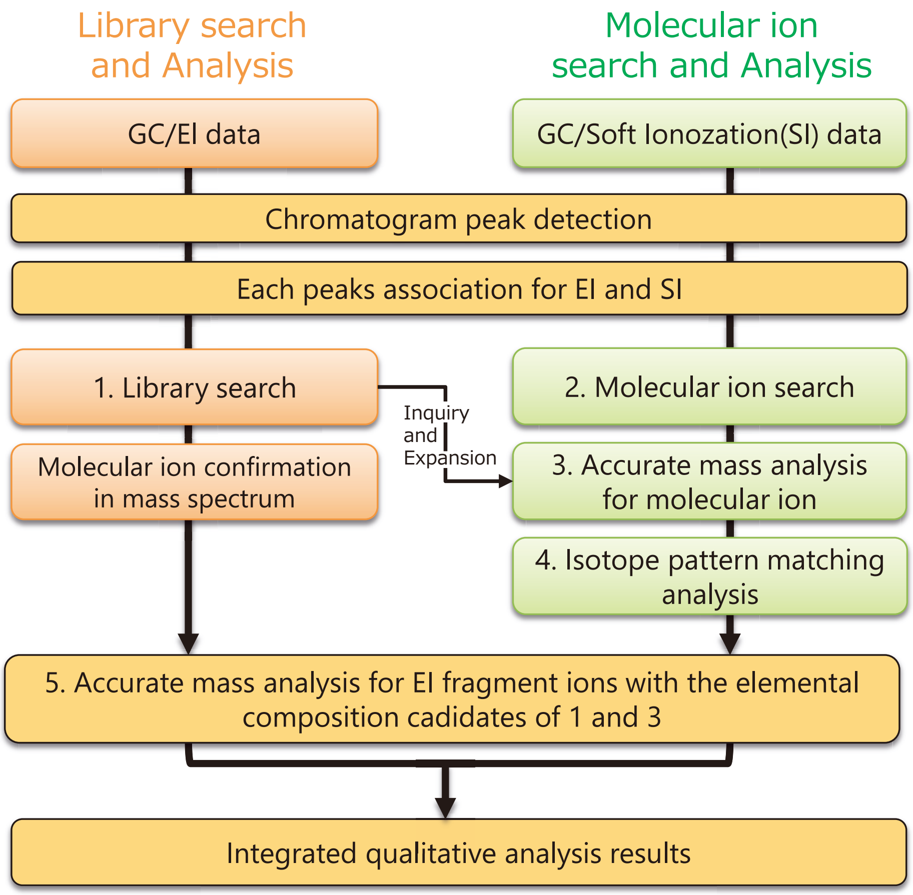
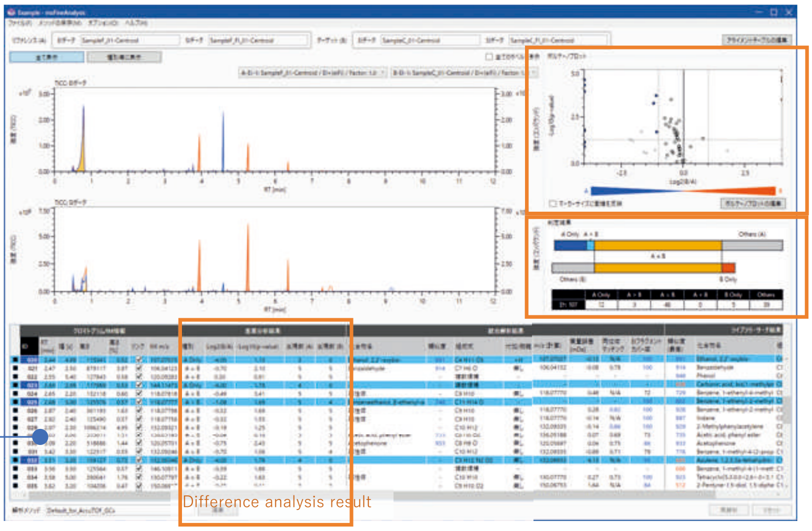

Library search and Analysis
Molecular ion search and Analysis
GC/EI data
GC/Soft Ionozation(SI) data
Chromatogram peak detection
Each peaks association for EI and SI
1. Library search
2. Molecular ion search
Inquiry and Expansion
Molecular ion confirmation in mass spectrum
3. Accurate mass analysis for molecular ion
4. Isotope pattern matching analysis
5. Accurate mass analysis for EI fragment ions with the elemental composition cadidates of 1 and 3
Integrated qualitative analysis results

Fig. 9 msFineAnalysis Ver.3: 2-samples comparison GUI



Volcano plot

- Difference of 2 samples and reproducibility
- Easy to view characteristic components

Classification

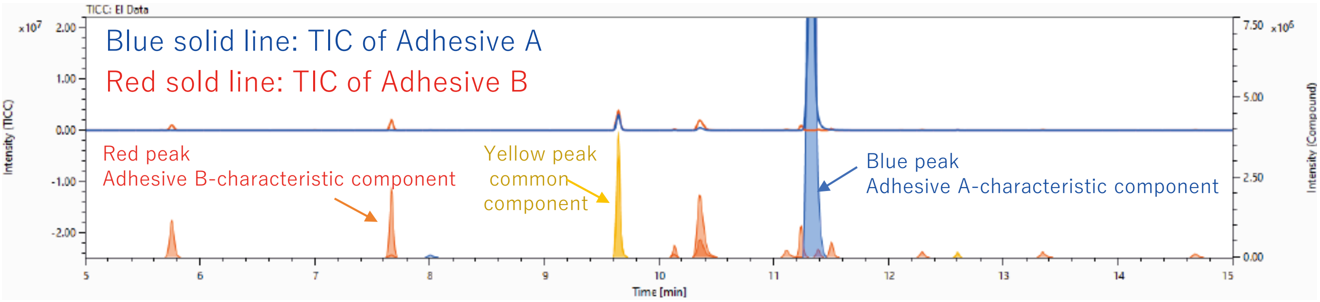
- Distribution of each class
- A or B only, A>B, A<B, A=B

Possible to rapidly find characteristic or common components to each component

EI/SI integrated analysis result

- For all the data ad detected components, identification judgement is conducted, and then, difference analysis that takes account of statistical reproducibility is performed.
- Difference analysis result: Classification, Intensity ratio (Log2(B/A), p value, Number of emergence, Volcano plot
- After difference analysis, the EI/SI integrated analysis of all the components is conducted.

Fig. 10 Evolved-gas difference analysis of material components using HS-GC-TOFMS



Blue solid line: TIC of Adhesive A

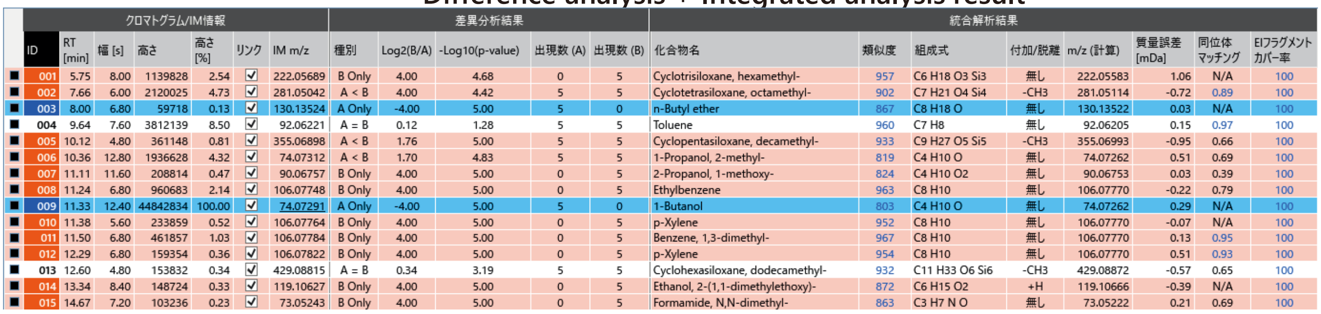
Red sold line: TIC of Adhesive B

Red peak
Adhesive B-characteristic component

Yellow peak
common component

Blue peak
Adhesive A-characteristic component

Difference analysis + Integrated analysis result



	クロマトグラム/IM情報							差異分析結果					統合解析結果							
	ID	RT [min]	幅 [s]	高さ	高さ [%]	リンク	IM m/z	種別	Log2(B/A)	-Log10(p-value)	出現数 (A)	出現数 (B)	化合物名	類似度	組成式	付加/脱離	m/z (計算)	質量誤差 [mDa]	同位体マッチング	EIフラグメントカバー率
■	001	5.75	8.00	1139828	2.54	☑	222.05689	B Only	4.00	4.68	0	5	Cyclotrisiloxane, hexamethyl-	957	C6 H18 O3 Si3	無し	222.05583	1.06	N/A	100
■	002	7.66	6.00	2120025	4.73	☑	281.05042	A < B	4.00	4.42	5	5	Cyclotetrasiloxane, octamethyl-	902	C7 H21 O4 Si4	-CH3	281.05114	-0.72	0.89	100
■	003	8.00	6.80	59718	0.13	☑	130.13524	A Only	-4.00	5.00	5	0	n-Butyl ether	867	C8 H18 O	無し	130.13522	0.03	N/A	100
■	004	9.64	7.60	3812139	8.50	☑	92.06221	A = B	0.12	1.28	5	5	Toluene	960	C7 H8	無し	92.06205	0.15	0.97	100
■	005	10.12	4.80	361148	0.81	☑	355.06898	A < B	1.76	5.00	5	5	Cyclopentasiloxane, decamethyl-	933	C9 H27 O5 Si5	-CH3	355.06993	-0.95	0.66	100
■	006	10.36	12.80	1936628	4.32	☑	74.07312	A < B	1.70	4.83	5	5	1-Propanol, 2-methyl-	819	C4 H10 O	無し	74.07262	0.51	0.69	100
■	007	11.11	11.60	208814	0.47	☑	90.06757	B Only	4.00	5.00	0	5	2-Propanol, 1-methoxy-	824	C4 H10 O2	無し	90.06753	0.03	0.39	100
■	008	11.24	6.80	960683	2.14	☑	106.07748	B Only	4.00	5.00	0	5	Ethylbenzene	963	C8 H10	無し	106.07770	-0.22	0.79	100
■	009	11.33	12.40	44842834	100.00	☑	74.07291	A Only	-4.00	5.00	5	0	1-Butanol	803	C4 H10 O	無し	74.07262	0.29	N/A	100
■	010	11.38	5.60	233859	0.52	☑	106.07764	B Only	4.00	5.00	0	5	p-Xylene	952	C8 H10	無し	106.07770	-0.07	N/A	100
■	011	11.50	6.80	461857	1.03	☑	106.07784	B Only	4.00	5.00	0	5	Benzene, 1,3-dimethyl-	967	C8 H10	無し	106.07770	0.13	0.95	100
■	012	12.29	6.80	159354	0.36	☑	106.07822	B Only	4.00	5.00	0	5	p-Xylene	954	C8 H10	無し	106.07770	0.51	0.93	100
■	013	12.60	4.80	153832	0.34	☑	429.08815	A = B	0.34	3.19	5	5	Cyclohexasiloxane, dodecamethyl-	932	C11 H33 O6 Si6	-CH3	429.08872	-0.57	0.65	100
■	014	13.34	8.40	148724	0.33	☑	119.10627	B Only	4.00	5.00	0	5	Ethanol, 2-(1,1-dimethylethoxy)-	872	C6 H15 O2	+H	119.10666	-0.39	N/A	100
■	015	14.67	7.20	103236	0.23	☑	73.05243	B Only	4.00	5.00	0	5	Formamide, N,N-dimethyl-	863	C3 H7 N O	無し	73.05222	0.21	0.69	100

Blue line: Adhesive A-characteristic component

Red line: Adhesive B-characteristic component

White line: Common component

msFineAnalysis AI Novel Qualitative Analysis Software for JMS-T2000GC with AI Structural Analysis

Ayumi Kubo MS Business Unit, JEOL Ltd.

JEOL developed msFineAnalysis as qualitative analysis software for our gas chromatograph time of flight mass spectrometer (GC-TOFMS). We implemented deconvolution detection, variance component analysis, and other features in the software through updates. We have recently developed a new version of the series called msFineAnalysis AI. msFineAnalysis AI is equipped with a structural analysis method using artificial intelligence (AI), called “AI structural analysis.” AI structural analysis enables the identification of molecular formulas as well as structural formulas of compounds that are not registered in the NIST 20 library (unknown compounds). The workflow of AI structural analysis is as stated below.

First, msFineAnalysis’s integrated analysis function identifies the molecular formula of an unknown compound. Next, based on the identified molecular formula, structural formula candidates are extracted from PubChem, the database containing over 100 million compounds. The AI predicts electron ionization (EI) mass spectra from the extracted structural formula candidates. Then, the structural formula candidates are ranked by comparing the predicted mass spectra with the measured mass spectrum. Finally, a candidate that ranks first is adopted as the analysis result.

Using the NIST 20 library, we trained the AI to predict mass spectra from structural formulas and evaluated its accuracy. From the results of accuracy evaluation, we confirmed that AI structural analysis is useful in the structural analysis of unknown compounds. In this report, we will introduce features of msFineAnalysis AI and provide our evaluation results.

Introduction

The electron ionization (EI) method is widely used as an ionization method for gas chromatograph mass spectrometry (GC-MS). Fragment ions are mainly observed in a mass spectrum obtained by the EI method (herein, an EI mass spectrum). Fragment ions reflect the structure of a compound and has a pattern unique to it. For this reason, in qualitative analysis of GC-MS, an EI mass spectrum is compared with libraries of EI mass spectra of reference compounds. The NIST library, the most widely used library of structural formulas and mass spectra, has about 300,000 registered compounds.

Meanwhile, PubChem, a major compound database, contains over 100 million substances as of 2023. However, EI mass spectra are not registered in PubChem. This means that most compounds in PubChem do not have EI mass spectral information, except for some also registered in the NIST library. When library searches are performed for EI mass spectra of such compounds, qualitative analysis results may not be obtained, or wrong compounds may possibly be identified. For these compounds that are not registered in the NIST 20 library, it is useful to combine [2] the field ionization (FI) and other soft ionization methods with a mass spectrometer [1] that obtains accurate mass. The specific procedure is as follows:

1. The EI and soft ionization mass spectra are compared, and a molecular ion peak is determined.
2. Based on the accurate mass of the determined peak, molecular formula candidates are obtained.
3. For obtained molecular formula candidates, isotope pattern analysis and accurate mass analysis of fragment ions in the EI mass spectrum are performed. Based on the results of these two analyses, the molecular formula is determined.

The above method is implemented in msFineAnalysis, which enables the automated identification of the molecular formula of an unknown compound. We have newly developed a structural analysis method using artificial intelligence (AI), called “AI structural analysis,” with an aim to obtain not only molecular formulas but also structural formulas of unknown compounds. The new version of msFineAnalysis equipped with AI structural analysis, msFineAnalysis AI, was introduced to the market in January 2023. In this article, we will provide an overview of AI structural analysis and report the results of its accuracy evaluation. In addition, we will show the results of applying this function to compounds that are not registered in the NIST 20 library.

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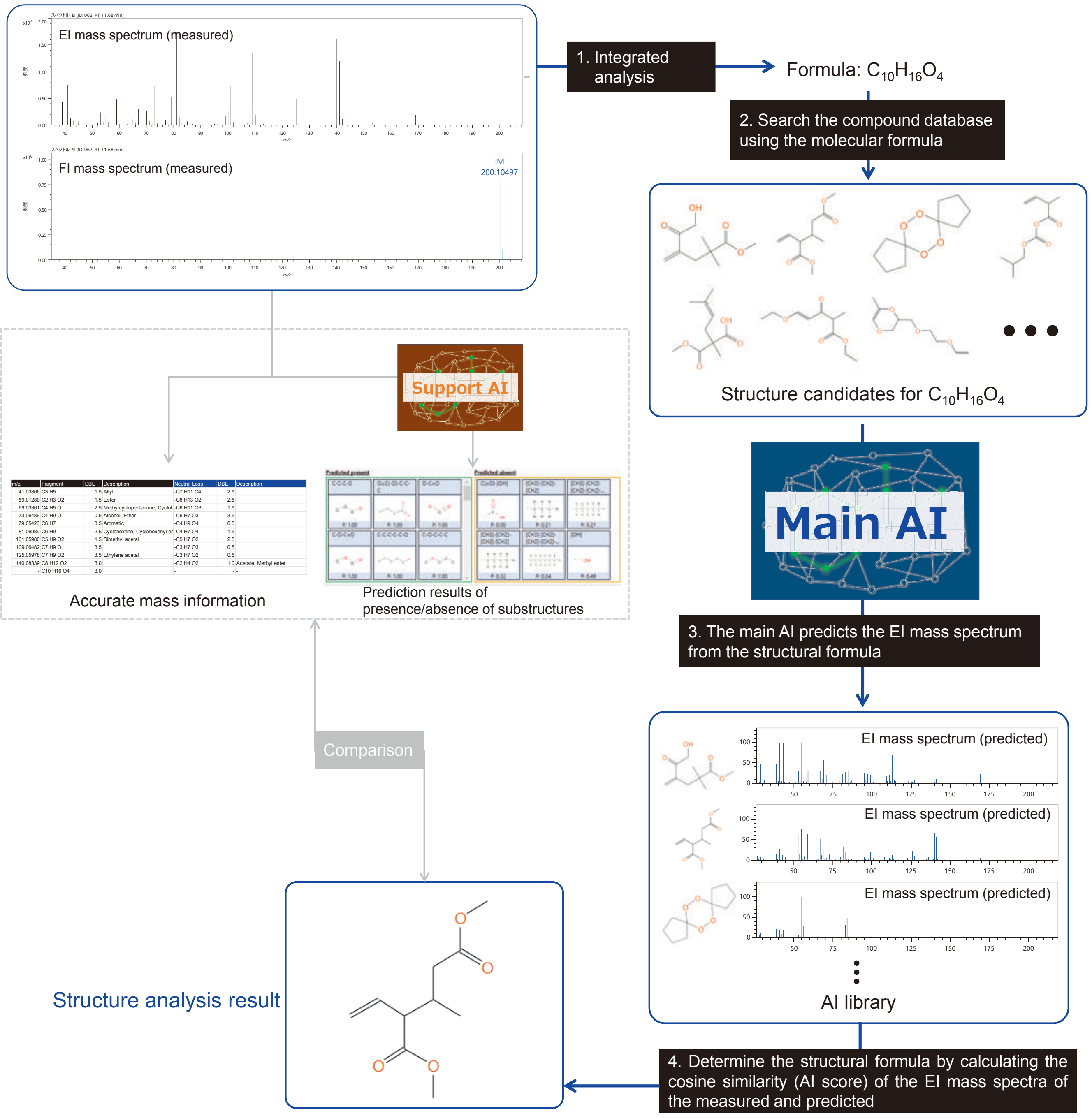
AI structural analysis

AI structural analysis uses two types of AI: main AI and support AI. **Figure 1** shows the procedures of integrated analysis and AI structural analysis for compounds that are not registered in the library. msFineAnalysis AI automatically performs the detection of a compound and steps 1 to 4 below. Details about two types of AI are described in the next section.

1. msFineAnalysis AI performs integrated analysis using the EI mass spectrum and the mass spectrum obtained by the FI method, a soft ionization method, to identify a molecular formula.
2. Based on the identified molecular formula, the software extracts structural formula candidates from PubChem database that contains over 100 million compounds. Ten thousand or less candidates are extracted.
3. The main AI predicts EI mass spectra for the extracted structural formula candidates.
4. By comparing the predicted EI mass spectra with the actual measured EI mass spectrum, the software ranks the structural formula candidates using AI scores (cosine similarities). Finally, the candidate that ranks first is adopted as the analysis result.

*The software displays the structural analysis results obtained through steps 1 to 4, as well as accurate mass information and

Fig. 1 Overview of AI structural analysis.


EI mass spectrum (measured)
FI mass spectrum (measured)
IM
200.10497
m/z
1. Integrated analysis
Formula: $C_{10}H_{16}O_4$
2. Search the compound database using the molecular formula
OH
O
Structure candidates for $C_{10}H_{16}O_4$
Support AI
Main AI
Predicted present
Predicted absent
Prediction results of presence/absence of substructures
3. The main AI predicts the EI mass spectrum from the structural formula
EI mass spectrum (predicted)
100
50
0
50
75
100
125
150
175
200
AI library
Comparison
Structure analysis result
4. Determine the structural formula by calculating the cosine similarity (AI score) of the EI mass spectra of the measured and predicted

m/z	Fragment	DBE	Description	Neutral Loss	DBE	Description
41.03866	C3 H5	1.5	Allyl	-C7 H11 O4	2.5	
59.01280	C2 H3 O2	1.5	Ester	-C8 H13 O2	2.5	
69.03361	C4 H5 O	2.5	Methylcyclopentanone, Cycloh	-C6 H11 O3	1.5	
73.06486	C4 H9 O	0.5	Alcohol, Ether	-C6 H7 O3	3.5	
79.05423	C6 H7	3.5	Aromatic	-C4 H9 O4	0.5	
81.06989	C6 H9	2.5	Cyclohexane, Cyclohexenyl es	-C4 H7 O4	1.5	
101.05980	C5 H9 O2	1.5	Dimethyl acetal	-C5 H7 O2	2.5	
109.06482	C7 H9 O	3.5		-C3 H7 O3	0.5	
125.05978	C7 H9 O2	3.5	Ethylene acetal	-C3 H7 O2	0.5	
140.08339	C8 H12 O2	3.0		-C2 H4 O2	1.0	Acetate, Methyl ester
-	C10 H16 O4	3.0		-	-	-

Accurate mass information

the results of partial structure prediction by the support AI. Analysts can use this information and knowledge to interpret the structural analysis results. However, this process is performed independently, and the structural analysis results can be automatically obtained without it.

Features of AI structural analysis include the EI mass spectrum prediction by main AI, as well as narrowing down candidates based on a molecular formula identified with integrated analysis. Before the measured mass spectrum is compared with AI-predicted EI mass spectra, the molecular formula identified by integrated analysis helps narrow down structural formula candidates. This allows the scope of structural formula candidates to be narrowed from 100 million to 10,000 or less, making it possible to perform an efficient and highly accurate structural analysis.

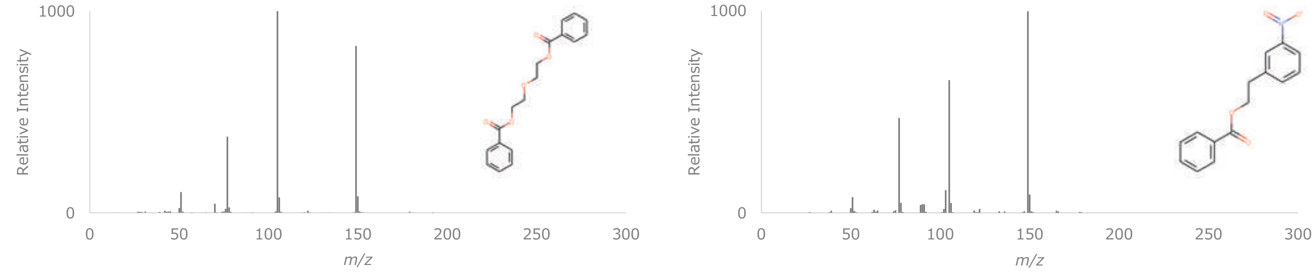
If a molecular formula is not identified in advance, the measured EI mass spectrum must be compared against the entire compound database, or must be narrowed down using compound species. In comparison against the entire database, the measured spectrum must be compared with 100 million EI mass spectra, resulting in a time-consuming and less accurate analysis. The reason for a lower accuracy is that some compounds are difficult to distinguish from others based on EI mass spectral information alone. The four compounds shown in **Fig. 2** have different structural and molecular formulas, but exhibit highly similar EI mass spectra. Therefore, only comparing their EI mass spectra is not sufficient for identification and may lead to wrong qualitative analysis results. Meanwhile, to identify compound species, information about samples and analysts' experience and knowledge are required. If there is not enough sample information, identifying compound species will be difficult. Additionally, an incorrect selection of species can lead to wrong structural analysis results. Consequently, analysis might be dependent on individual skills of analysts, resulting in a low reproducibility. On the other hand, AI structural analysis generates correct analysis results for the four compounds shown in Fig. 2, because it narrows down structural formula candidates beforehand using the molecular formula identified by integrated analysis as mentioned earlier.

msFineAnalysis AI is not equipped with the main AI. Instead, it is equipped with the "AI library," which contains structural formulas extracted from PubChem and mass spectra predicted from the structural formulas by the main AI. The AI library helps eliminate the need for mass spectrum prediction during analysis, improving the analysis throughput. After an analyst selects measurement data and presses the button to start the analysis, msFineAnalysis AI automatically performs all the processing to complete the structural analysis. The analyst can obtain structural analysis results for 100 compounds within 10 minutes. The AI library also eliminates the need for connecting to the compound database via the Internet during analysis, enabling a stable and stand-alone analysis.

Figure 3 shows the graphical user interface (GUI) of AI structural analysis. Structural formulas are listed in descending order of AI score at the lower part of the window. On the top left corner of the list is the structural analysis result. As the information about the structural formula, its IUPAC name and PubChem CID (identification number in PubChem database) are also displayed. The number of structural formula candidates for the molecular formula and the histogram created using AI score are displayed on the upper right of the window. These various kinds of information help the analyst see the whole picture of the structural analysis results.

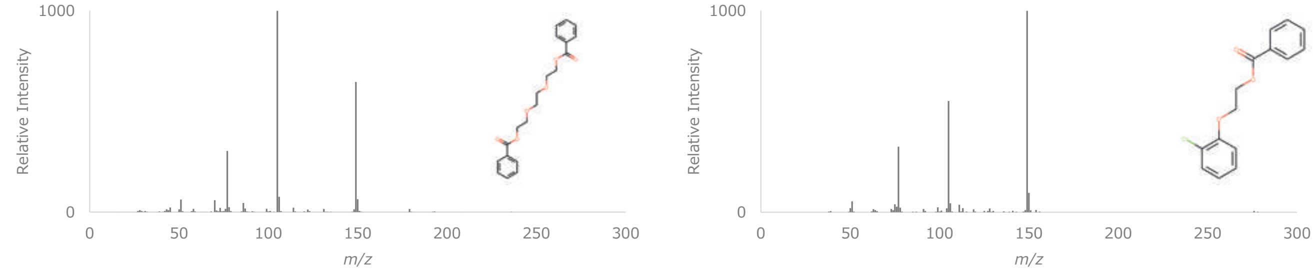
In addition, if there is knowledge about the target compound, the analyst can filter structural formulas using partial structures such as benzene ring and methyl ester. When the analyst presses the button on the right edge of the window, it displays the mass spectrum and information for accurate mass as well as the prediction results of partial structures performed by the support AI. The analyst can confirm and interpret the structural analysis results.

Fig. 2 EI mass spectra of four compounds registered in the NIST 20 library.



a) Diethylene glycol dibenzoate [$C_{18}H_{18}O_5$]

b) Benzoic acid, 2-(3-nitrophenyl)ethyl ester [$C_{15}H_{13}NO_4$]



c) 2,2'-(Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) dibenzoate [$C_{20}H_{22}O_6$]

d) Benzoic acid, 2-(2-chlorophenoxy)ethyl ester [$C_{15}H_{13}ClO_3$]

Two types of AI

This section describes two types of AI used in AI structural analysis.

The main AI employs Graph Convolutional Networks (GCN) [3], a type of deep learning, as its model (**Fig. 4**, top). GCN operates as follows: First, the machine searches structural formulas for partial structures that produce signals characteristic of a mass spectrum, and generates a lot of partial structures. Then, the machine predicts a mass spectrum based on the generated partial structural information (Fig. 4, bottom).

The specific processing is as follows: First, the structural formula is converted to graph data before being input into GCN (**Fig. 5**). In graph data, atoms and bonds in the structural formula are treated as nodes and edges, respectively. In addition, nodes hold information on the elemental species of atoms, and edges hold information on the type of bonds, as their feature vector. For example, a node for the carbon atom has the feature vector (1, 0, 0, ...), a node for the oxygen atom has the feature vector (0, 1, 0, ...), and a node for the nitrogen atom has the feature vector (0, 0, 1, ...).

Next, the machine performs convolutions on the structural formula that was converted to graph data as shown in the top left of Fig. 4. Through convolutions, each node sifts through and obtains information on neighboring nodes and edges. The machine learns to recognize the connection of atoms as a block by repeating convolutions.

Then, the machine performs pooling of each atom as shown in the top right of Fig. 4. This enables the machine to grasp the characteristics of the structural formula and predict a mass spectrum.

The support AI employs the traditional machine learning (regression) instead of deep learning. The machine predicts the presence or absence of 48 partial structures from ions and neutral loss based on the accurate-mass mass spectra (**Fig. 6**). The support AI is simple and uses dozens of coefficients. Therefore, the machine can provide prediction results and their characteristic peaks at the same time.

Accuracy evaluation of AI structural analysis

— Accuracy evaluation of EI mass spectrum prediction —

AI structural analysis uses mass spectra that are predicted from the structural formulas by the main AI. The main AI was trained using the structural formulas and mass spectra of 270,000 compounds, which account for 90% of the NIST 20 library data. During training, the weight of the main AI was optimized so that patterns of mass spectra predicted from the structural formulas match those of mass spectra in the NIST 20 library. Out of the remaining 30,000 compounds, 10,000 were allocated for validation to prevent overfitting, and 20,000 were used to evaluate the accuracy of EI mass spectrum prediction.

We evaluated the accuracy of the main AI's EI mass spectrum

Fig. 3 GUI of AI structural analysis.

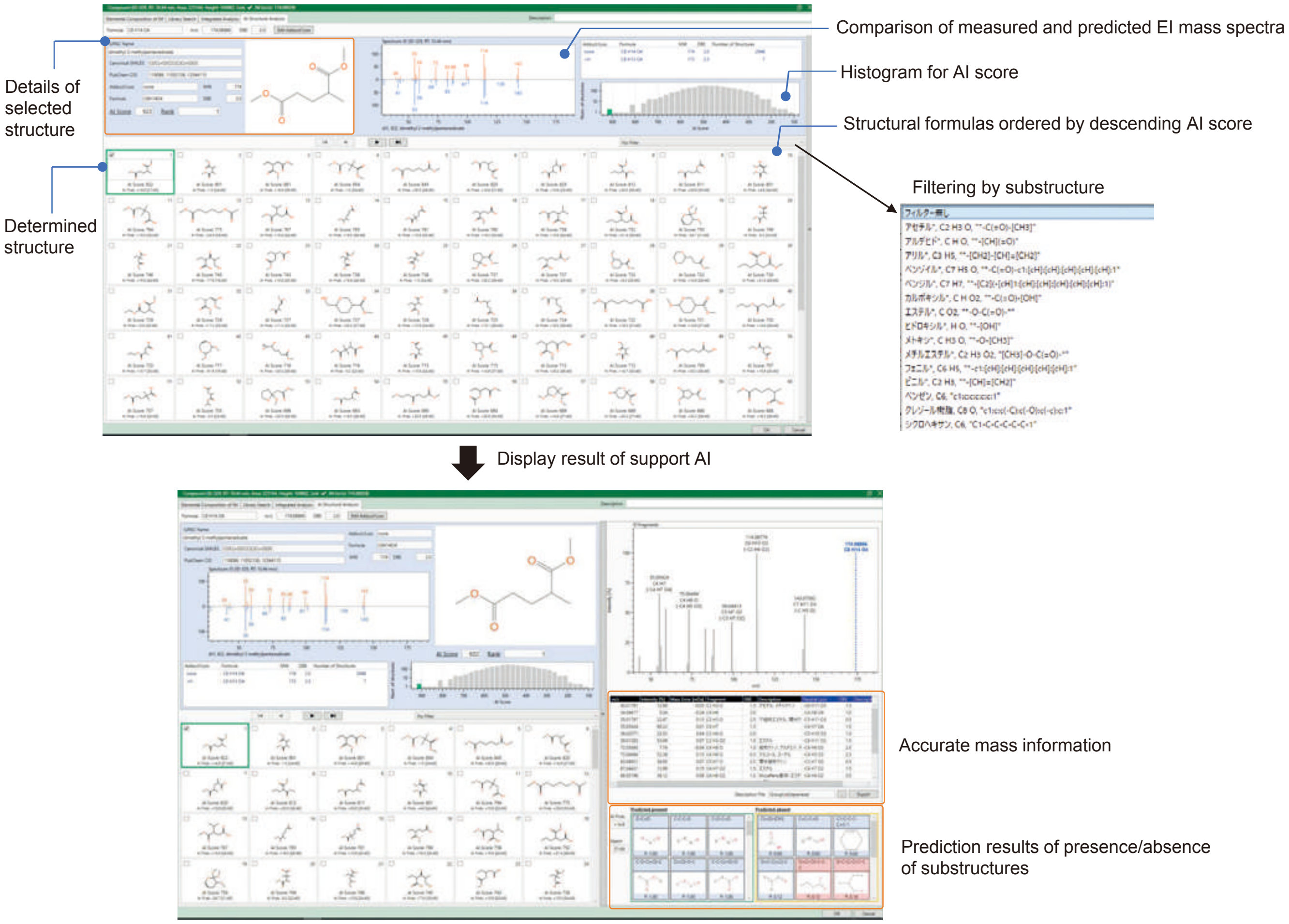

Details of selected structure
Determined structure
Comparison of measured and predicted EI mass spectra
Histogram for AI score
Structural formulas ordered by descending AI score
Filtering by substructure
フィルター無し
アセチル*, C2 H3 O, "*-C(=O)-[CH3]"
アルデヒド*, C H O, "*-[CH](=O)"
アリル*, C3 H5, "*-[CH2]-[CH]=[CH2]"
ベンゾイル*, C7 H5 O, "*-C(=O)-c1:[cH]:[cH]:[cH]:[cH]:[cH]:1"
ベンジル*, C7 H7, "*-[CH2](-[cH]1:[cH]:[cH]:[cH]:[cH]:[cH]:1)"
カルボキシル*, C H O2, "*-C(=O)-[OH]"
エステル*, C O2, "*-O-C(=O)-*"
ヒドロキシル*, H O, "*-[OH]"
メトキシ*, C H3 O, "*-O-[CH3]"
メチルエステル*, C2 H3 O2, "[CH3]-O-C(=O)-*"
フェニル*, C6 H5, "*-c1:[cH]:[cH]:[cH]:[cH]:[cH]:1"
ビニル*, C2 H3, "*-[CH]=[CH2]"
ベンゼン, C6, "c1:c:c:c:c:c:1"
クレゾール骨格, C8 O, "c1:c:c(-C):c(-O):c(-c):c:1"
シクロヘキサン, C6, "C1-C-C-C-C-C-1"
Display result of support AI
Accurate mass information
Prediction results of presence/absence of substructures

Fig. 4 Graph Convolutional Networks used in the main AI.

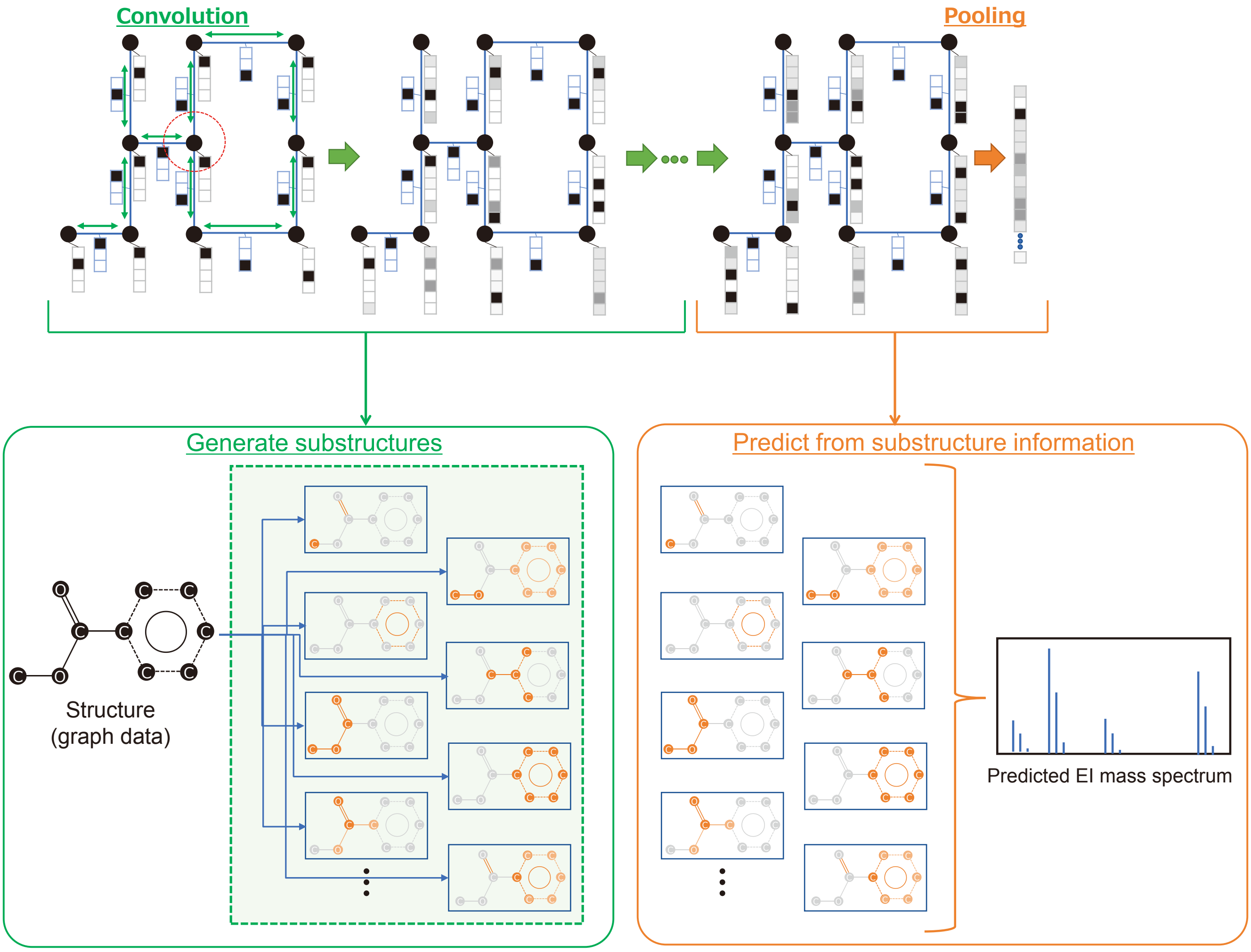
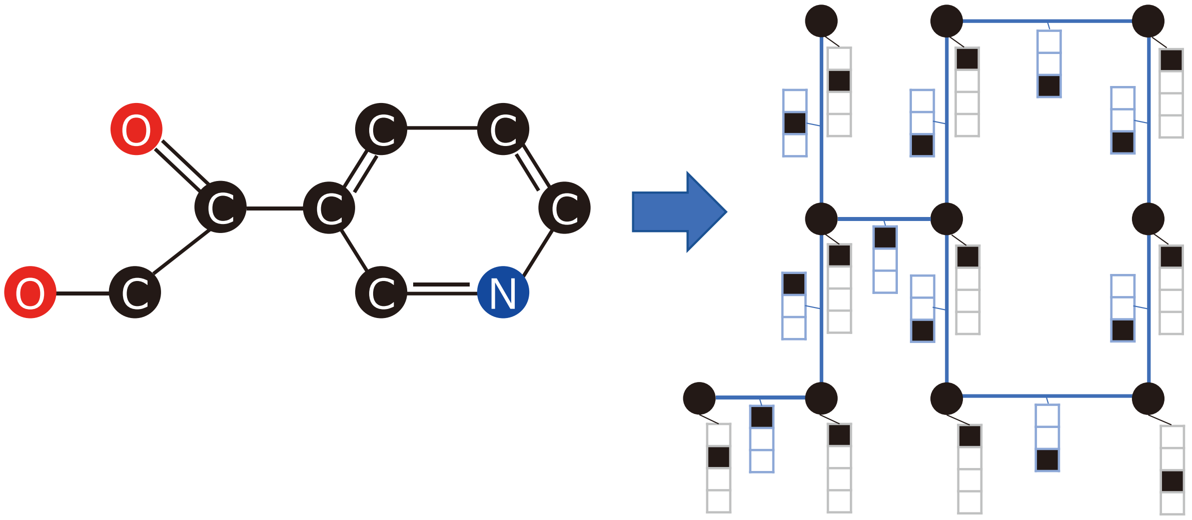

Convolution
Pooling
Generate substructures
Structure
(graph data)
Predict from substructure information
Predicted EI mass spectrum

Fig. 5 Conversion of a structural formula to graph data.


O
O
C
C
C
C
C
C
C
N

prediction using 20,000 compounds that were not used in training. In the evaluation, the trained main AI predicted EI mass spectra from the structural formulas of the target compounds. We used the cosine similarity between the predicted EI mass spectrum and an EI mass spectrum registered in the NIST 20 library as the index of accuracy evaluation. A cosine similarity of 1 means the two EI mass spectra match perfectly. As the cosine similarity is closer to 0, they match less.

Figure 7 shows a histogram of cosine similarities calculated using 20,000 compounds. The histogram shows that more than 90% of the compounds had a cosine similarity of over 0.4. In addition, the 0.7-0.8 segment had the highest number of the compounds. The average cosine similarity was 0.72. We confirmed that the main AI can reproduce mass spectra with a high accuracy by predicting them from the structural formulas.

Figure 8 shows as examples the comparison between the measured and predicted EI mass spectra for each of the compounds with above-average, near-average, and below-average cosine similarities. For Benzamide, 3-methyl-N-decyl-, which had an above-average cosine similarity, the EI mass spectrum was reproduced almost completely including mass peaks with low intensity. The reason is thought to be that this compound consists of only benzene rings, alkane chains, and amide groups, many of which are registered in the NIST 20 library. For N-Acetyl-3-(3-formyl-4-methoxyphenyl)-d-alanine methyl ester, which had a near-average cosine similarity, mass peaks with relatively high intensity were reproduced, and the overall patterns were similar. This compound has a somewhat complex structure, with multiple side chains attached to a benzene ring, compared with the structural formula of Benzamide, 3-methyl-N-decyl-. This is thought to be why a complete mass spectrum was not reproduced. For Cyclododecane, 1,5,9-tris(acetoxy)-, which had a below-average cosine similarity, the overall pattern was not well reproduced. A possible reason is that this compound includes a large 12-membered ring, and the NIST 20 library contains a small number of compounds that have this ring. This may have prevented the machine to be trained enough. However, some mass peaks, including the most intense one at *m/z* 43, were reproduced.

Fig. 6 Overview of the support AI.

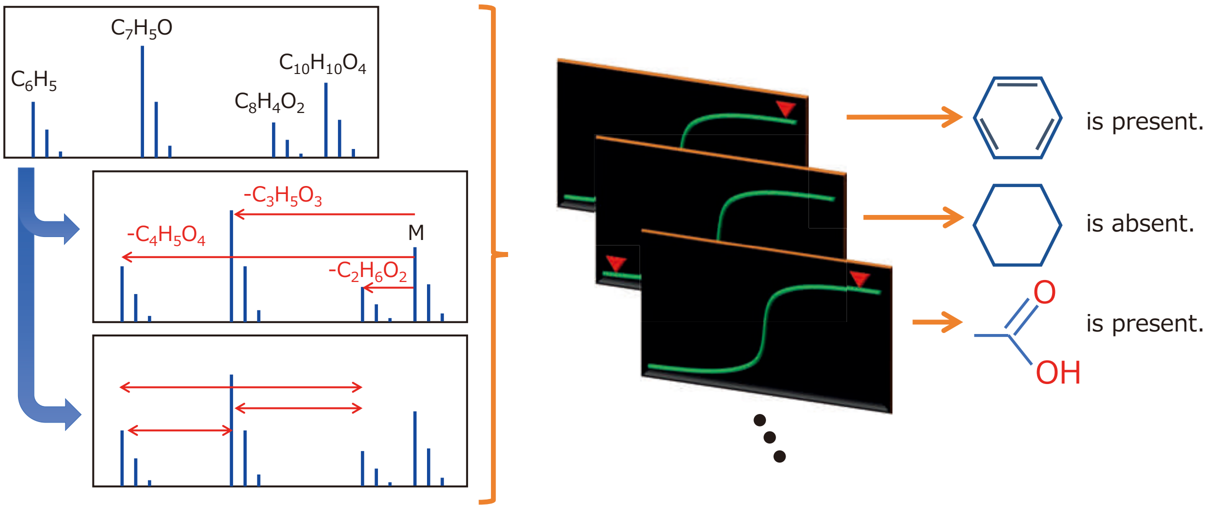


Fig. 7 Histogram of cosine similarities calculated using 20,000 compounds that were not used in training.

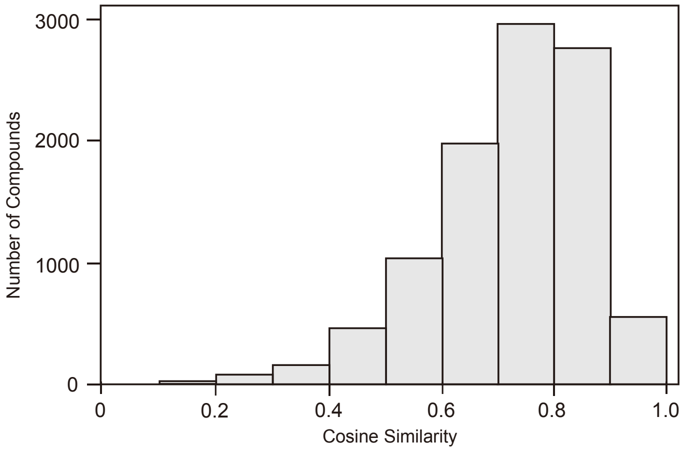
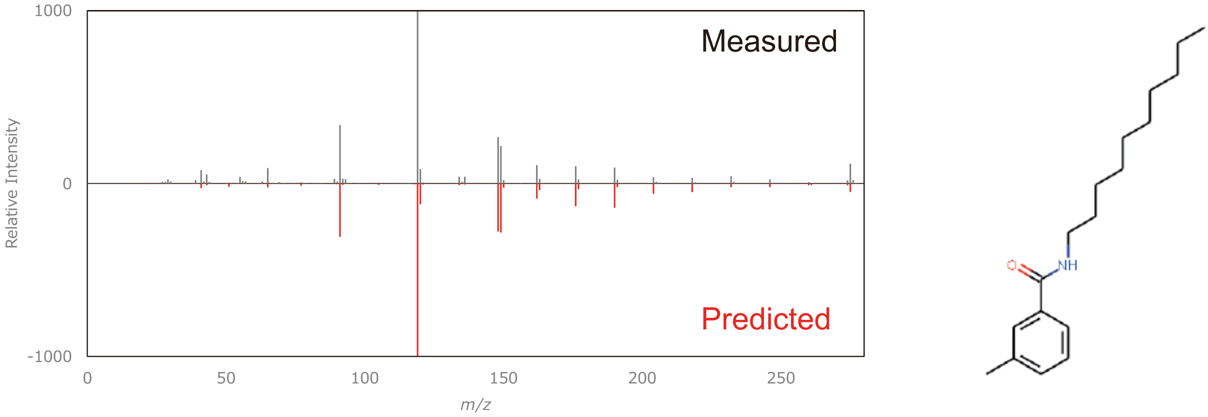
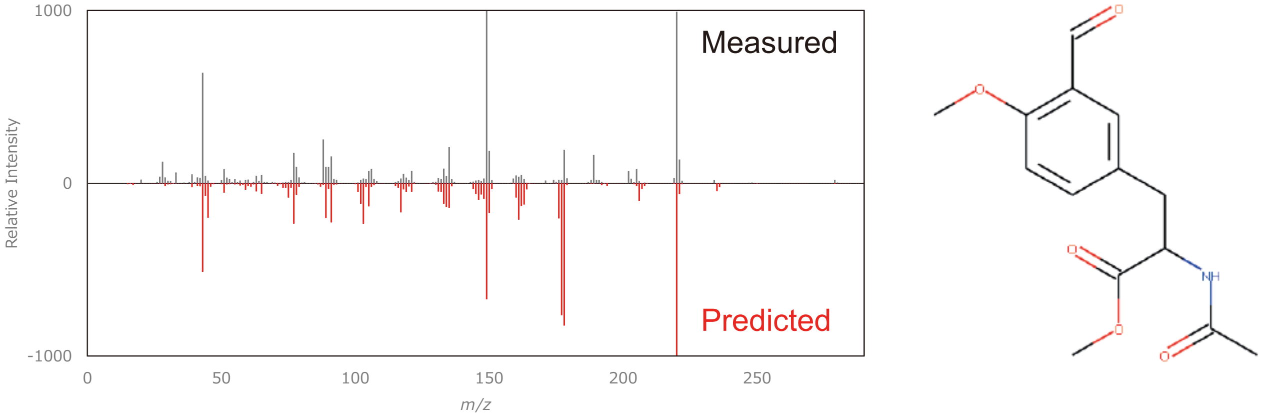


Fig. 8 Comparison between the measured and predicted EI mass spectra.

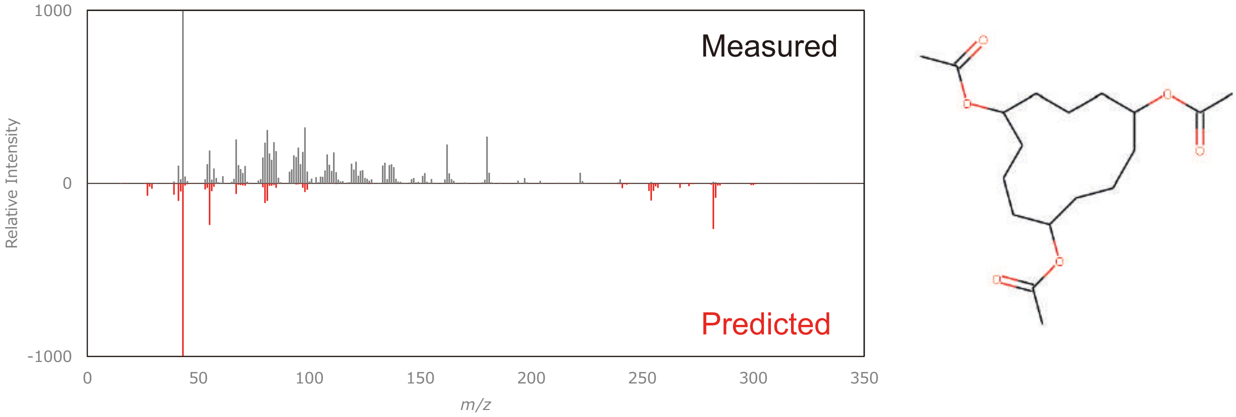
a) benzamide, 3-methyl-N-decyl-
[cosine similarity: 0.95]



b) N-Acetyl-3-(3-formyl-4-methoxyphenyl)-d-alanine methyl ester
[cosine similarity: 0.72]



c) cyclododecane, 1,5,9-tris(acetoxy)-
[cosine similarity: 0.34]



— Accuracy evaluation of structural analysis —

AI structural analysis compares EI mass spectra predicted from structural formula candidates with the actual measured EI mass spectrum to identify the structural formula. We evaluated the accuracy of this structural formula identification. The evaluation method is as follows: First, for the compounds in the NIST 20 library that were not used in training, structural formulas (compounds) that have the same molecular formula were extracted from the compound database. Next, the trained main AI predicted EI mass spectra for the correct structural formula and the extracted ones. The predicted EI mass spectra were compared with the ones registered in the NIST 20 library, and based on their cosine similarities, all the structural formulas, including the correct one, were ranked. We used the rank given by the correct structural formula among all the structural formulas as the index of accuracy evaluation. In this evaluation, to set certain criteria, we used only molecular formulas for which at least 100 compound candidates were extracted from the compound database.

Table 1 shows the results of ranking structural formulas for 14,581 compounds. The results indicate that the correct structural formula ranked top for 22% of the compounds. In addition, the correct structural formula ranked in the top 1% for 73% of the compounds. Ranking in the top 1% means that the correct structural formula was placed within the top 10 out of 1,000 candidates. The PubChem compound database contains many compounds that have quite similar structural formulas. With taking this into consideration, this structural formula identification method is said to be highly accurate.

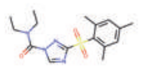
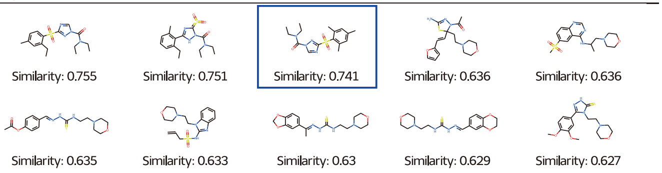
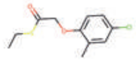
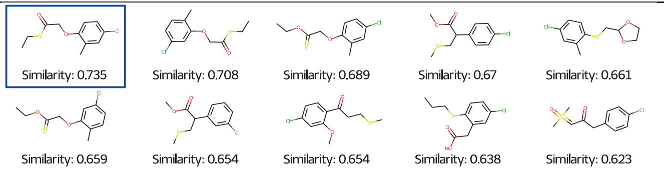
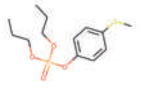
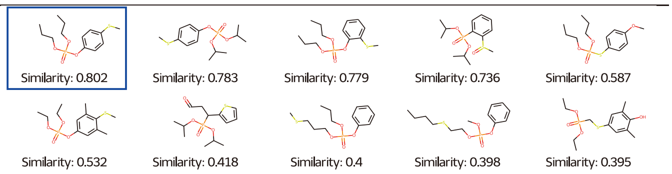
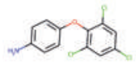
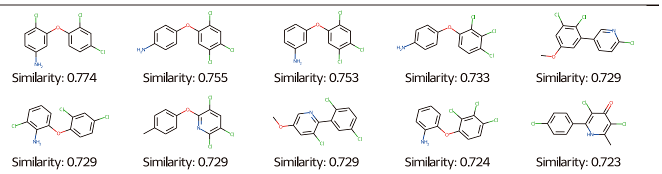
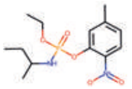
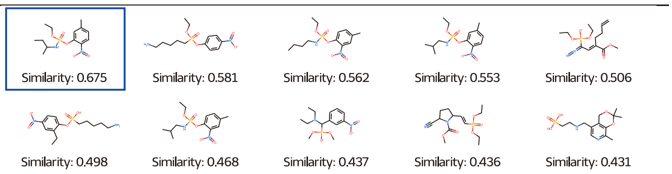
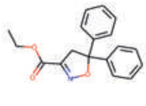
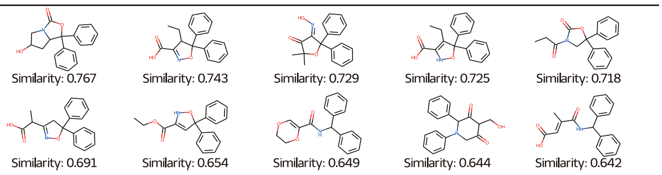
Next, we evaluated the effectiveness of this method for completely unknown compounds. We used model compounds that are not registered in the NIST 20 library to perform the evaluation. The following model compounds were used: Cafenstrole (CAS: 125306-83-4, Wako), MCPA-thioethyl (CAS: 25319-90-8, Wako), Propaphos (CAS: 7292-16-2, Wako), CNP-amino (CAS: 26306-61-6 Wako), Butamifos oxon (CAS: 56362-05-1 Wako), and Isoxadifen-ethyl (CAS: 163520-33-0, Wako).

The measured EI mass spectra for the model compounds were prepared by measuring standard samples. **Table 2** shows

Table 1 Results of accuracy evaluation on 14,581 compounds.

	Top	Within the top 1%	Within the top 5%	Within the top 10%
Number of Compounds	3215 (22 %)	10618 (73 %)	12934 (89 %)	13594 (93 %)

Table 2 Examples of structural analysis results.

Compound Name	Structure	Similarity	Rank	Top 10 structures
Cafenstrole		0.741	3 (2933)	 Similarity: 0.755 Similarity: 0.751 Similarity: 0.741 Similarity: 0.636 Similarity: 0.636 Similarity: 0.635 Similarity: 0.633 Similarity: 0.63 Similarity: 0.629 Similarity: 0.627
MCPA-thioethyl		0.735	1 (729)	 Similarity: 0.735 Similarity: 0.708 Similarity: 0.689 Similarity: 0.67 Similarity: 0.661 Similarity: 0.659 Similarity: 0.654 Similarity: 0.654 Similarity: 0.638 Similarity: 0.623
Propaphos		0.802	1 (27)	 Similarity: 0.802 Similarity: 0.783 Similarity: 0.779 Similarity: 0.736 Similarity: 0.587 Similarity: 0.532 Similarity: 0.418 Similarity: 0.4 Similarity: 0.398 Similarity: 0.395
CNP-amino		0.710	14 (618)	 Similarity: 0.774 Similarity: 0.755 Similarity: 0.753 Similarity: 0.733 Similarity: 0.729 Similarity: 0.729 Similarity: 0.729 Similarity: 0.729 Similarity: 0.724 Similarity: 0.723
Butamifos oxon		0.675	1 (56)	 Similarity: 0.675 Similarity: 0.581 Similarity: 0.562 Similarity: 0.553 Similarity: 0.506 Similarity: 0.498 Similarity: 0.468 Similarity: 0.437 Similarity: 0.436 Similarity: 0.431
Isoxadifen-ethyl		0.586	22 (5348)	 Similarity: 0.767 Similarity: 0.743 Similarity: 0.729 Similarity: 0.725 Similarity: 0.718 Similarity: 0.691 Similarity: 0.654 Similarity: 0.649 Similarity: 0.644 Similarity: 0.642

the rank given by the correct structural formula, its score, and top 10 structural formulas in descending order of score for each model compound. For three compounds out of the six, the correct structural formula ranked top. For Isoxadifen-ethyl, the correct structural formula ranked lowest compared with the other five model compounds. However, it was placed 22nd out of 5,348 candidates, within top 1%. The result suggests that this structural formula identification method is effective in narrowing down the correct structural formula from many candidates. The top-ranked structural formulas for Cafenstrole, CNP-amino, and Isoxadifen-ethyl have the same size and number of rings as their correct structural formulas do, and they show considerable similarity. The results of our evaluation on these six compounds reveal that this identification method is useful in structural analysis. **Figure 9** shows the comparisons between the measured and predicted mass spectra. The measured and predicted mass spectra exhibit the same peaks with high intensity, although they are different in detailed peak intensities and distributions of mild peaks.

These results confirm that this method is effective in the structural analysis of unknown compounds.

Conclusions

Previous msFineAnalysis software features integrated analysis based on accurate mass measurement and molecular ion observation using the soft ionization method, which are features of the JMS-T2000GC. Integrated analysis enables the identification of molecular formulas of unknown compounds. The new version, msFineAnalysis AI, is equipped with structural analysis using artificial intelligence (AI), which enables molecular formulas as well as structural formulas to be automatically obtained. msFineAnalysis AI extracts structural formula candidates based on the molecular formulas identified by integrated analysis. Then, it uses the EI mass spectra predicted from the structural formula candidates by the AI to identify the structural formula. The combination of integrated analysis and AI enables a highly efficient and accurate structural analysis. All the processes are performed automatically and offline, leading to a stable analysis.

References

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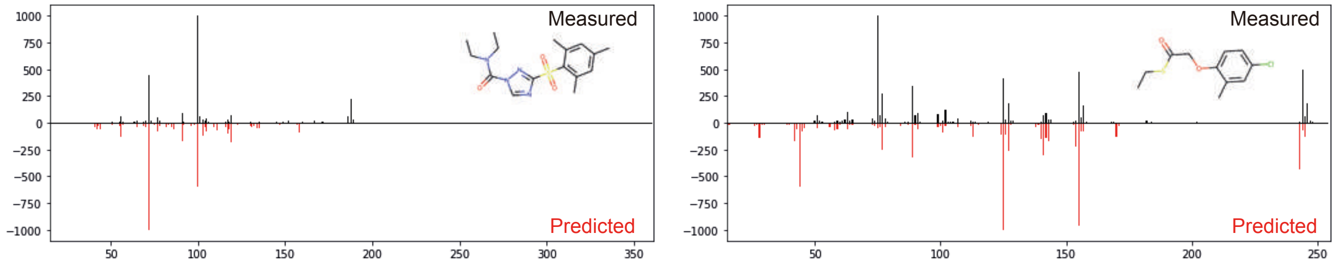
[2] Masaaki Ubukata, Yoshihisa Ueda. Development of an Integrated Analysis Method for the JMS-T200GC High Mass-Resolution GC-TOFMS by Electron Ionization and Soft Ionization Methods. *JEOL news* Vol. **54**.

[3] J. Gilmer, S. S. Schoenholz, P. F. Riley, O. Vynyals, G. E. Dahl. Neural message passing for Quantum chemistry. Proceedings of the 34th International Conference on Machine Learning. 2017;70:1263-1272.

Fig. 9 Comparison between the measured and predicted mass spectra.

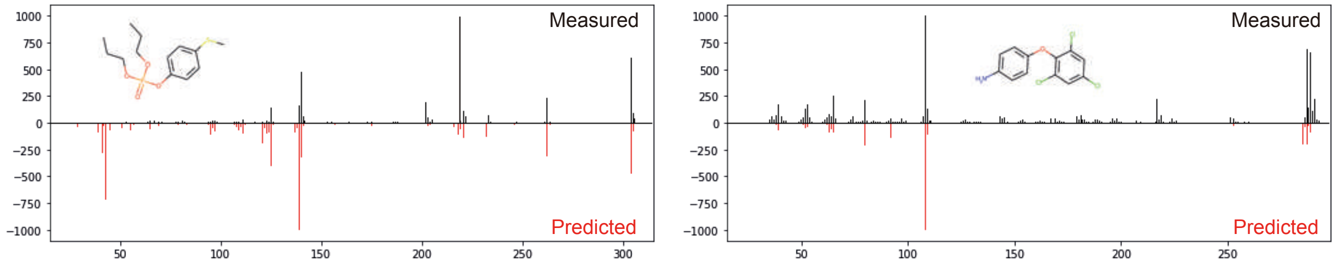
a) Cafenstrole [cosine similarity: 0.741]

b) MCPA-thioethyl [cosine similarity: 0.735]



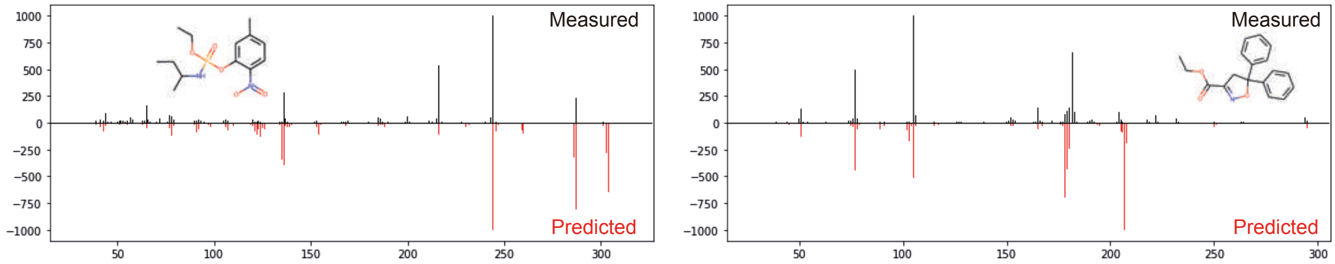
c) Propaphos [cosine similarity: 0.802]

d) CNP-amino [cosine similarity: 0.710]



e) Butamifos oxon [cosine similarity: 0.675]

f) Isoxadifen-ethyl [cosine similarity: 0.586]



Development of an Integrated Analysis Method for the JMS-T200GC High Mass-Resolution GC-TOFMS by Electron Ionization and Soft Ionization Methods

Masaaki Ubukata and Yoshihisa Ueda MS Business Unit, JEOL Ltd.

We have developed a new software package with a new workflow for identifying unknown compounds from GC-high resolution MS (GC-HRMS) data. In this newly designed workflow, unknown compounds are determined by correlating the data results obtained by using a hard ionization method such as Electron Ionization (EI) and a soft ionization method such as Field Ionization (FI) with high-resolution mass spectrometry.

The new data reduction software 'msFineAnalysis' was used to identify unknown compounds in the data from pyrolysis/GC-HRMS analysis of vinyl acetate resin. The new workflow implemented in this software improved the accuracy of identification and eliminated misidentification of unknown compounds.

Introduction

When a sample composed of unknown compounds is analyzed by using GC-MS (gas chromatograph mass spectrometer), the compound peaks separated by the GC are identified by using their corresponding mass spectra. Electron Ionization (EI) is widely used for GC-MS [1] because of the availability of mass spectral databases that contain hundreds of thousands of spectra for compounds that are frequently analyzed by GC-MS. EI mass spectra are typically acquired using the standard ionizing electron energy of 70 eV which produces fragmentation patterns that are directly related to the compound's structure. Additionally, the relative intensity ratios for the fragment peaks are always constant if the ionizing electron energy is kept constant. Consequently, the mass spectrum of the same compound acquired with the same ionization energy, exhibits a reproducible spectral pattern. Mass spectral database searches (commonly called "library searches") utilize this feature of EI for compound identification [2].

While mass spectral library searches are very simple to implement for identifying compounds acquired by GC-MS, this method has the following disadvantages. 1) For compounds having similar structures (in particular for compounds whose elemental compositions of substituents are slightly different from each other), the mass spectra acquired by using EI can be very similar, and therefore, the chemical compounds may not be identified from the library search result. 2) For compounds which are not included in the library search databases (i.e. pyrolyzed products detected by pyrolysis analysis), it is impossible to identify them with a library search. In case 1), if the molecular ion peaks are found in the mass spectra, it can be possible to determine the elemental composition of the target compound. However, because EI is a high-energy ionization method, the chemical structure of a given compound can fragment extensively, making it difficult, if not impossible, to observe the molecular ions. If a low-resolution mass spectrometer is used for the measurement, even if molecular ions are observed, their mass-to-charge ratios are only reported as integer values. An integer value for the molecular ion *m/z* is not sufficient for determining the elemental composition because there are many combinations of elemental masses that can have a specific integer mass. A high-resolution mass spectrometer (HRMS) can provide accurate mass measurements with a precision in the 3rd or 4th decimal place which in turn allows for more accurate elemental composition assignments. For case 2), identification of chemical compounds that are not present in the databases cannot be obtained by library searches alone so using the HRMS accurate mass information for molecular ions (as well as fragment ions) can help narrow down the possibilities.

In this work, we used the JMS-T200GC AccuTOF^{TM} GCx-plus GC-high resolution time-of-flight MS (GC-HRTOFMS) equipped with both EI and Soft Ionization (SI) to generate molecular ions. We then used the newly developed software application 'msFineAnalysis' to combine the information obtained from the EI library searches with the SI molecular ion accurate mass and isotope analysis to identify the sample components [3, 4]. The details of the msFineAnalysis qualitative analysis work flow will be introduced along with the application of this software to the thermal analysis of resin products.

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Analysis Flow

Figure 1 shows the qualitative analysis work flow using only library search by the conventional EI method (left) and using the new integrated analysis work flow utilized in the msFineAnalysis software (right). The procedures for the new analysis work flow are as follows:

1. For the data acquired by the EI and SI methods, the peaks in the total ion chromatogram (TIC) are detected to create mass spectra.
2. For the mass spectra acquired by each ionization method, each spectrum is associated with a corresponding chromatographic peak retention time, and then, the mass spectra with the same retention times are assigned as the same component.
3. The EI mass spectra are library searched for matches. ①
4. The SI mass spectra are used to determine the molecular ions for each analyte. ②
5. An accurate mass analysis is done for each of the detected molecular ions. This analysis involves directly comparing the possible elemental composition formulas to the statistically-significant EI library search results from Step 3 in order to narrow down the most likely elemental composition candidates for a given analyte. ③
6. An isotope pattern analysis is conducted to further refine the molecular ion elemental composition candidates. ④
7. Using these possible molecular ion composition formulas as the search constraints, an accurate mass analysis is performed for the fragment ions in the EI mass spectra. If a given molecular ion formula is not correct, then the EI fragment ions will not show good matches and will result in a low EI fragment ion interpretation ratio.
8. The interpretation ratio is used to further refine the molecular-ion formula candidates. ⑤
9. Finally, all of these analysis results are integrated together into a qualitative analysis report for the peaks detected in the sample. ⑥

Analysis Examples: Pyrolysis data analysis of vinyl acetate resin

(1) Analytical Condition

A JMS-T200GC equipped with a pyrolyzer was used to measure a commercially-available vinyl acetate resin. A combination EI and Field Ionization (FI) source was used with the system to measure the samples. The measurement conditions for the GC-HRTOFMS and pyrolyzer are listed in **Table 1**. Since an EI/FI combination ion source was used for this work, it was not necessary to exchange sources when switching between the EI and FI methods.

The EI and FI sample measurements were subjected to the new msFineAnalysis work flow. Afterwards, these analysis results were compared to an analysis with only EI library search results to confirm the improved capabilities of this new, innovative analysis work flow.

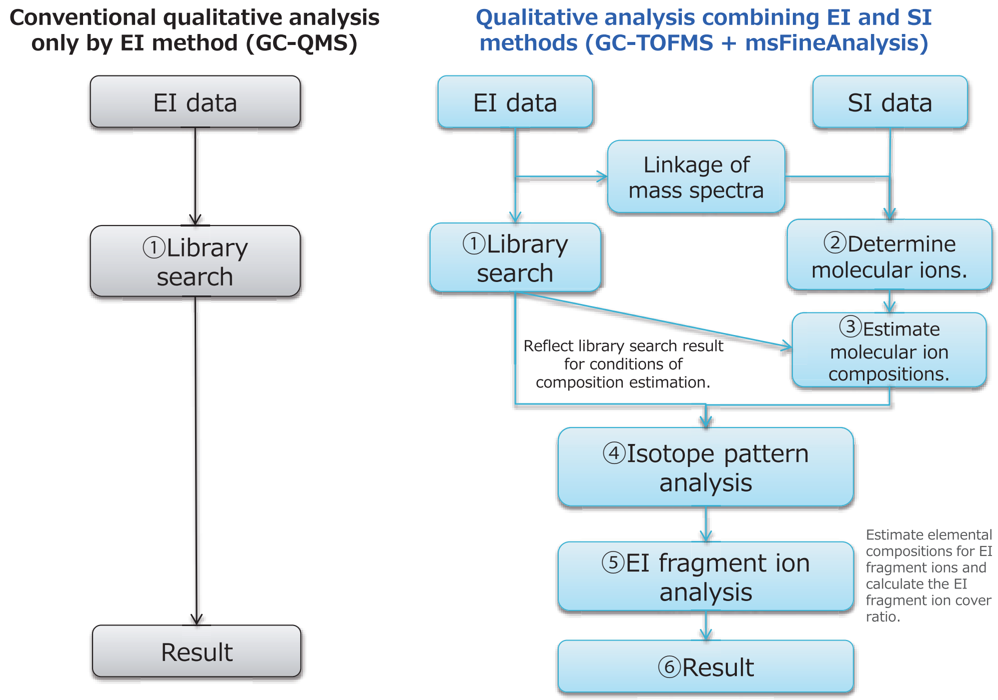
(2) Analysis Results

The EI and SI total ion current chromatograms (TICCs) for the resin sample were automatically analyzed by msFineAnalysis using the analysis work flow shown in Fig. 1. In total, 33 components were detected during the sample measurement (**Fig. 2**). Each component was categorized according to the reliability of its identification results. The three classification types are shown as different colors in the report (**Fig. 3**).

Green:	EI search result had high similarity score and matched the identified SI molecular ion. High probability that the analyte has been identified correctly.
Orange:	Multiple candidates with significant molecular elemental composition formulas were obtained.
White:	No significant molecular composition formula was obtained.

A comparison between the integrated analysis results acquired by msFineAnalysis and the conventional GC/EI analysis

Fig. 1 Analysis flow comparison with conventional GC-MS qualitative analysis


Conventional qualitative analysis only by EI method (GC-QMS)
EI data
①Library search
Result
Qualitative analysis combining EI and SI methods (GC-TOFMS + msFineAnalysis)
EI data
SI data
Linkage of mass spectra
①Library search
②Determine molecular ions.
Reflect library search result for conditions of composition estimation.
③Estimate molecular ion compositions.
④Isotope pattern analysis
⑤EI fragment ion analysis
Estimate elemental compositions for EI fragment ions and calculate the EI fragment ion cover ratio.
⑥Result

results are shown in **Fig. 4**. Conventional EI data analysis only resulted in the high-confidence identification of one-fourth of the components. However, the new msFineAnalysis work flow, which also included molecular ion formula estimation and isotopic analysis, enabled the determination of more than 90% of the components. Furthermore, the new software used the EI fragment ion formulas to obtain additional structural information for the analytes. For the library-registered components (similarity: high), the new analysis method showed highly reliable qualitative analysis results that involved combining the library search results and the molecular composition formulas. Even for unknown components that do not show a good library match (similarity: low) and are thus difficult to identify by using the conventional GC/EI method (left in Fig. 1), msFineAnalysis also estimated the molecular composition formulas obtained with soft ionization. Consequently, the new analysis method, irrespective of high or low similarity, uses the accurate mass molecular ion information to estimate elemental compositions that can further refine the candidate identification. Furthermore, these results showed that msFineAnalysis provides a very effective work flow for the qualitative analysis of GC-MS data.

To demonstrate the details of the new analysis method, we will present two kinds of identification examples that are typical for qualitative analysis.

(3) Example 1: Peaks detected at a retention time around 2.49 min

Mass spectra acquired by EI and FI are shown in **Fig. 5**. A library search of the EI mass spectra resulted in several matches. However, all of the matching spectra had similarity scores of less than 700, indicating that there is a low probability that the related peaks correspond to these compounds. On the other hand, the SI mass spectra showed clear peaks at *m/z* 106.06 and *m/z* 128.12. The *m/z* 128.12 (larger *m/z*) was selected as the

Table 1 Measurement Condition

[Pyrolysis condition]	
Pyrolysis Temperature	600 ℃
[GC Condition]	
Column	DB-5msUI, 15 m × 0.25 mm, 0.25 μm
Oven Temperature	50 ℃ (1 min) - 30 ℃ /min - 330 ℃ (1.7 min)
Injection Mode	Split mode (100:1)
[MS condition]	
Spectrometer	JMS-T200GC (JEOL Ltd.)
Ion Source	EI/FI combination ion source
Ionization	EI+ : 70 eV, 300 μA FI+ : -10 kV, 6 mA/10 msec, Carbotec emitter

Fig. 2 Total Ion current chromatograms for Py-GC/TOFMS analysis of vinyl acetate sample

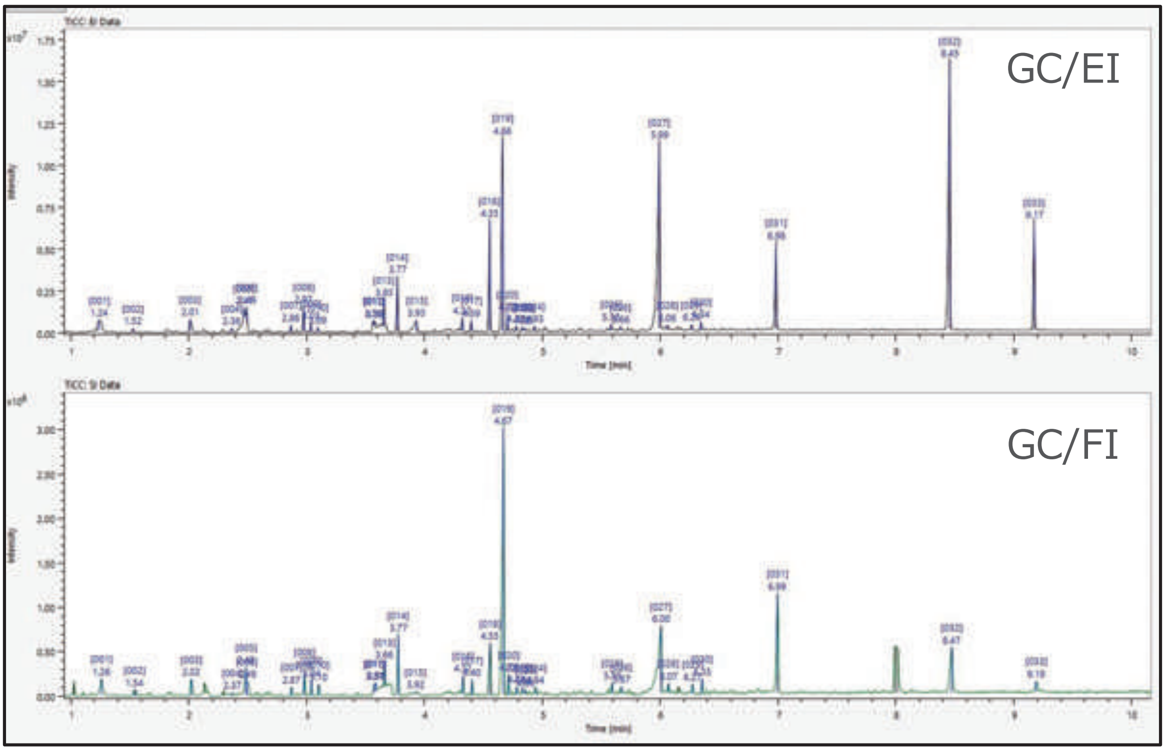
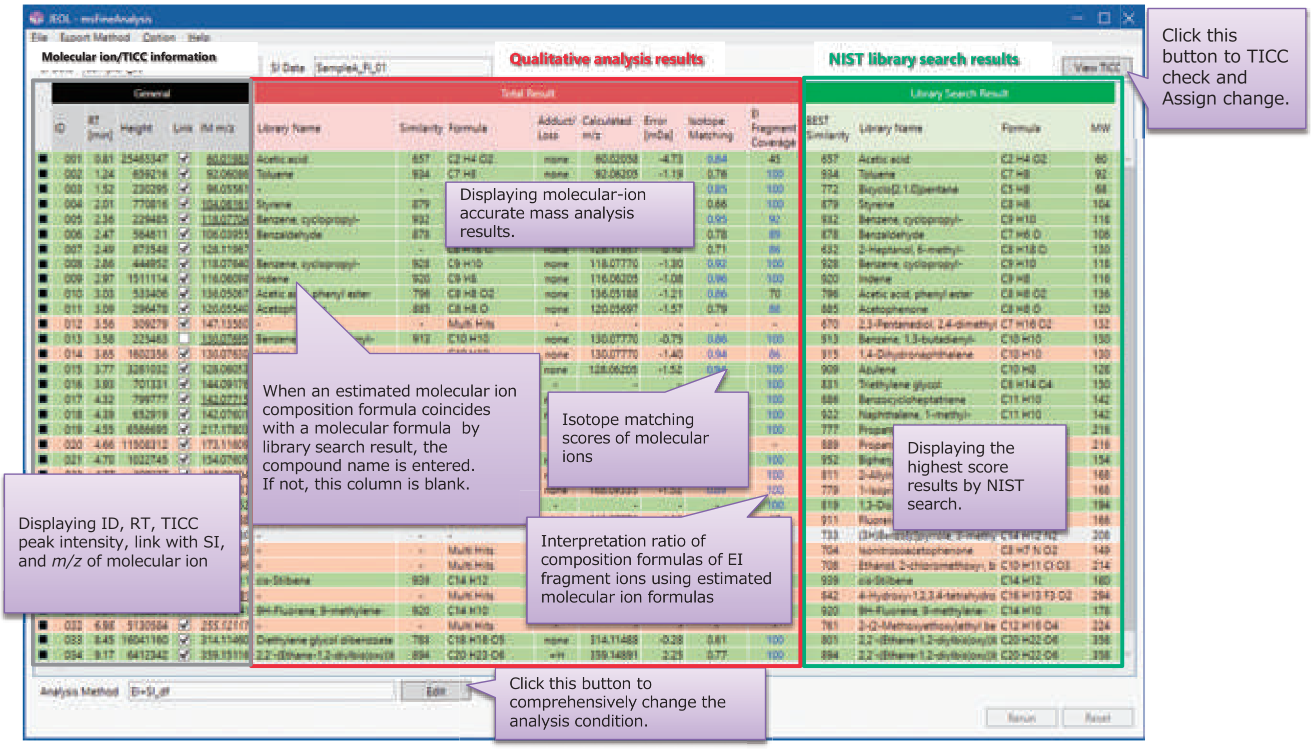


Fig. 3 Data analysis results of vinyl acetate sample by msFineAnalysis software


JEOL - msFineAnalysis
File Export Method Option Help
Molecular ion/TICC information
SI Data SampleA_FI_01
Qualitative analysis results
NIST library search results
View TICC
General
Total Result
Library Search Result
ID
RT [min]
Height
Link
M m/z
Library Name
Similarity
Formula
Adduct/Loss
Calculated m/z
Error [mDa]
Isotope Matching
EI Fragment Coverage
BEST Similarity
Library Name
Formula
MW
Analysis Method
EI+SI_df
Edit
Rerun
Reset

Click this button to TICC check and Assign change.

Displaying molecular-ion accurate mass analysis results.

When an estimated molecular ion composition formula coincides with a molecular formula by library search result, the compound name is entered. If not, this column is blank.

Isotope matching scores of molecular ions

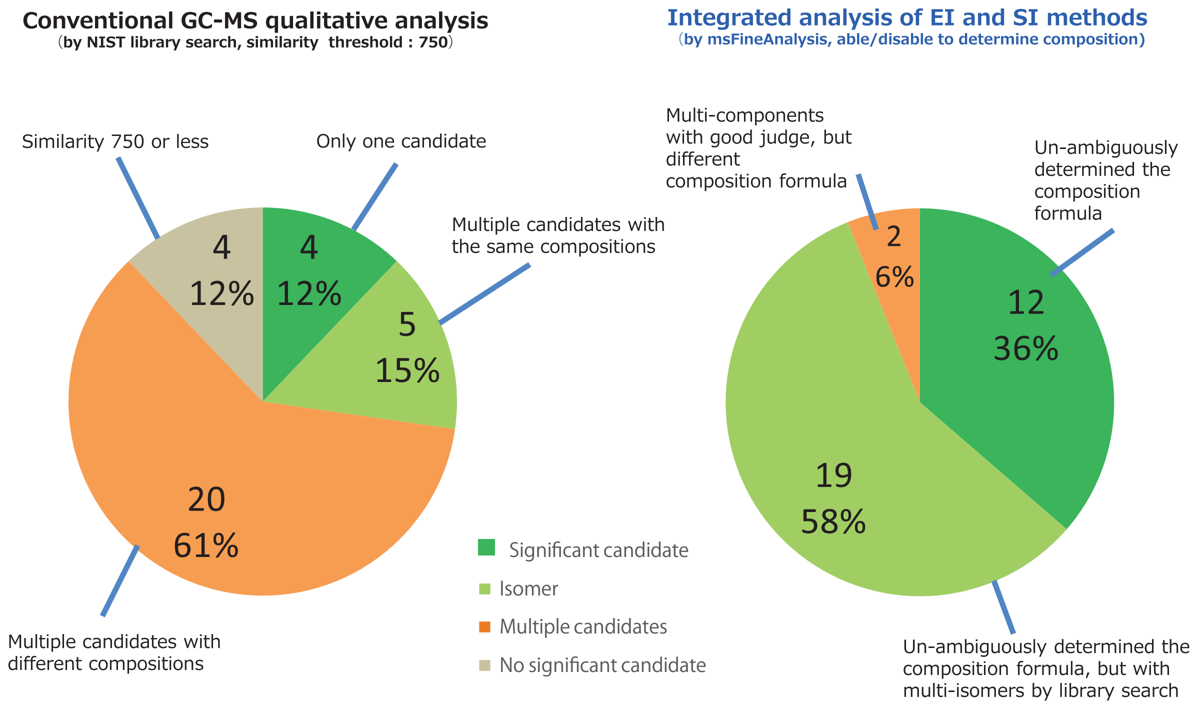
Displaying the highest score results by NIST search.

Displaying ID, RT, TICC peak intensity, link with SI, and *m/z* of molecular ion

Interpretation ratio of composition formulas of EI fragment ions using estimated molecular ion formulas

Click this button to comprehensively change the analysis condition.

Fig. 4 Comparison of number of peaks that were identified between conventional data analysis flow and new work flow with msFineAnalysis software



Conventional GC-MS qualitative analysis
(by NIST library search, similarity threshold : 750)

Integrated analysis of EI and SI methods
(by msFineAnalysis, able/disable to determine composition)

molecular ion peak, and its accurate mass was used to estimate an elemental composition of $C_8H_{15}O$ (error 0.1 mDa). Next, this composition "$C_8H_{15}O$" was used as the element limits to estimate the fragment ion formulas in the EI mass spectrum. The results showed that 6 out of the 7 higher intensity peaks were likely a product of the "$C_8H_{16}O$" elemental composition. These results also suggested that 1 of the 7 peaks may not be a peak derived from $C_8H_{16}O$. To explore this possibility, extracted ion current chromatograms (EICC) for both peak types ($C_8H_{16}O$:*m/z* 55.05, 97.10 and non-$C_8H_{16}O$:*m/z* 75.04) were created, and the results showed that there was a clear difference in the chromatographic peak shapes/times in each chromatogram (**Fig. 6**). These results suggested that ion peaks produced by different chemical species were co-eluting at a retention time of ~2.49 min. To test this hypothesis, a library search was conducted using only a mass spectrum produced from the latter part of the peak at 2.49 min, so as to exclude as much as possible the contribution from the co-eluted components. The search came up with "3-Penten-1-ol, 2,2,4-trimethyl- : $C_8H_{16}O$" with a similarity of over 800. Furthermore, the composition for this compound exactly matched the initially-estimated elemental composition of $C_8H_{16}O$.

The peak at 2.49 min analyzed by using the conventional GC/EI method (only library search of the EI mass spectra) did not provide enough information to narrow down the candidate compounds. However, the msFineAnalysis work flow using the combined EI and FI mass spectral information resulted in a single, strongly supported candidate "3-Penten-1-ol, 2,2,4-trimethyl-".

(4) Example 2: Peaks detected at a retention time around 8.15 min

Mass spectra acquired by EI and FI are shown in **Fig. 7**. A library search of the EI mass spectrum revealed one compound with a similarity of 801 (2,2'-(Ethane-1,2-diylbis(oxy)) bis(ethane-2,1-diyl) dibenzoate) along with 7 other compounds with similarities of more than 750 (shown on **Table 2**). Consequently, it was difficult to make an unambiguous identification using only the library search results. The SI mass spectra showed a clear peak appearing at *m/z* 315.12, suggesting that this peak is likely to be the molecular ion. **Table 3** shows the possible elemental composition formulas calculated using the accurate mass of this peak. All four compositions had calculated values that were within 3 mDa of the measured value. Next, a comparison of Table 2 and Table 3 showed that "Diethylene glycol dibenzoate" (similarity of 788) had an elemental composition that matched $C_{18}H_{18}O_5$. Next, this composition "$C_{18}H_{18}O_5$" was used as the element limits to estimate the fragment ion formulas for 5 high intensity peaks in the EI mass spectrum. All of the peaks produced elemental compositions (errors less than 3 mDa) consistent this compound (**Table 4**). These results all strongly support "Diethylene glycol dibenzoate" as the peak detected at retention time ~8.45 min.

As described above, the peak detected at a retention time around 8.45 min was difficult to identify through an EI library search result alone, as there were a number of possible candidates with close similarity matches. However, the msFineAnalysis software, with its ability to combine EI and SI data analysis, resulted in a single, strongly supported candidate "Diethylene glycol dibenzoate".

Summary

For the analysis of unknown compounds using GC-TOFMS with high-mass resolution, a new analytical work flow was devised that combined conventional EI library search, molecular ion elemental compositions acquired using a SI method (FI for this work), and the EI fragment ion accurate mass information to identify the targeted chemical species. This innovative analysis work flow led to the development of the new GC-HRMS analysis software msFineAnalysis.

Fig. 5 Mass spectra at around retention time 2.49 min measured by EI and FI

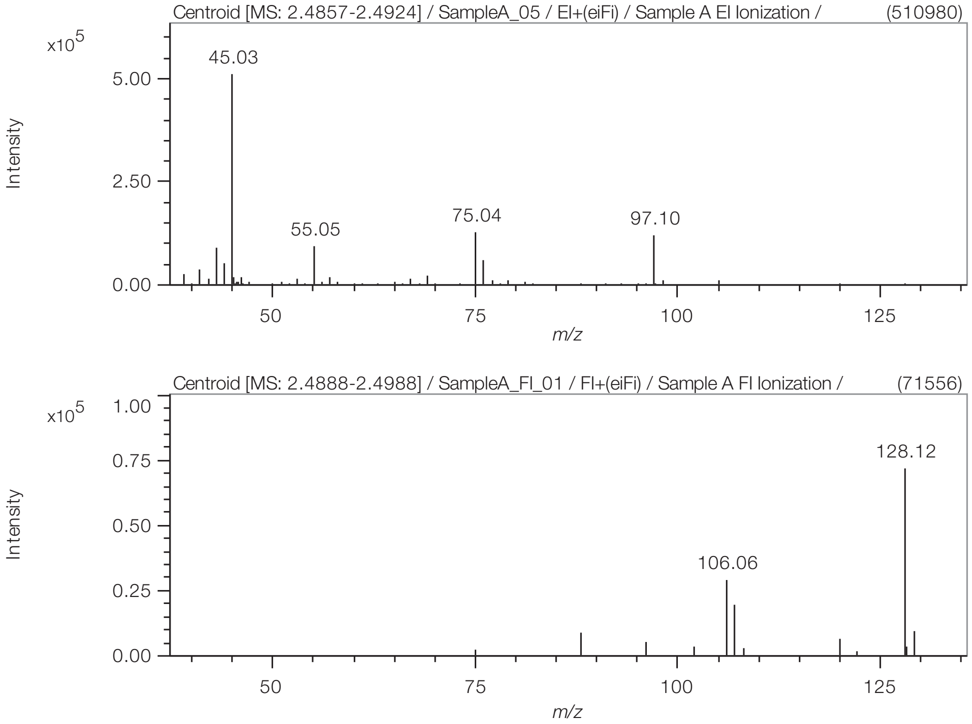


Fig. 6 TICC and EICC at around retention time 2.49 min

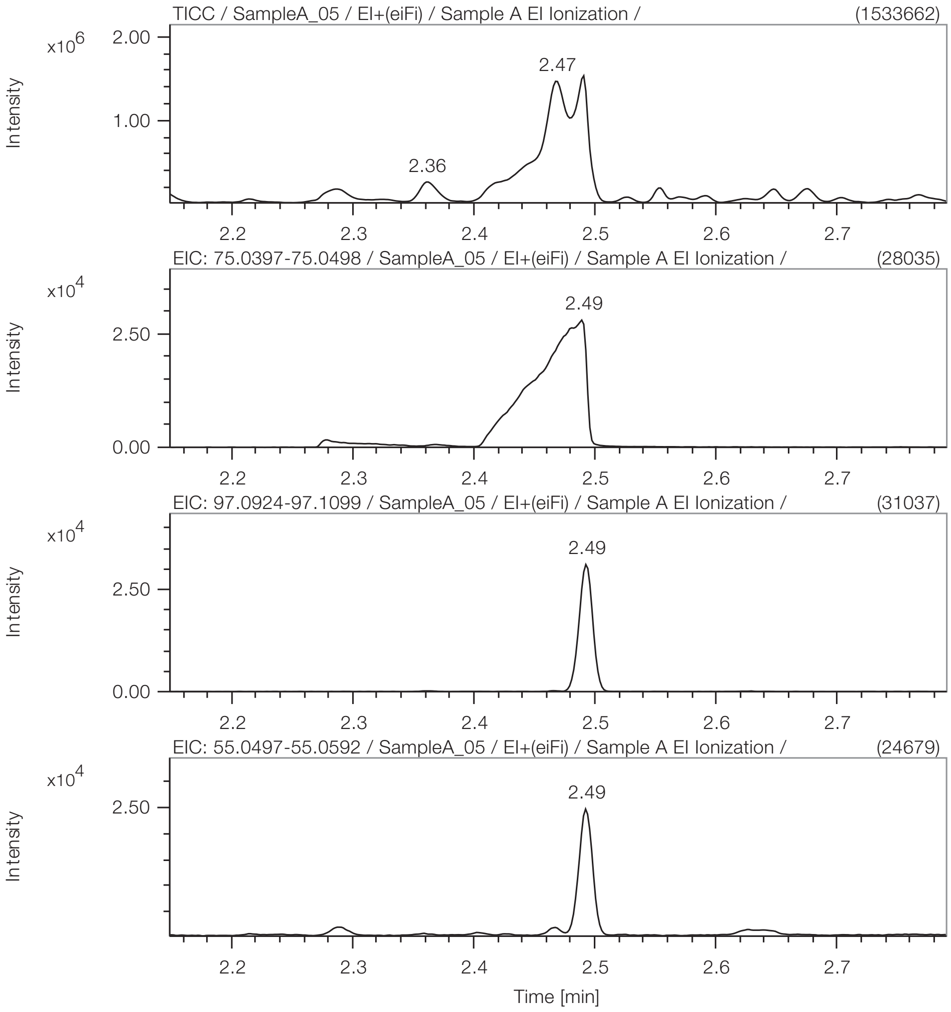


Fig. 7 Mass spectra at around retention time 8.45 min measured by EI and FI

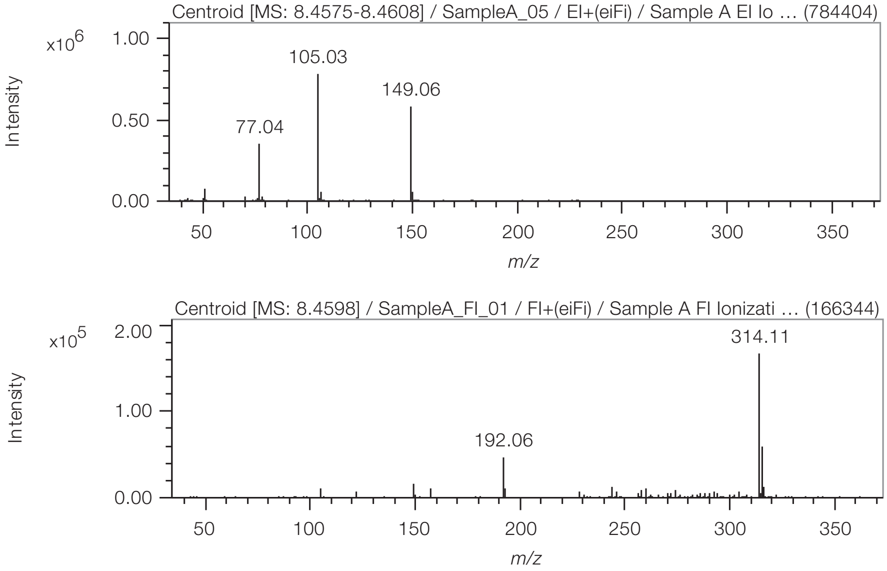


Table 2 Results of library search for the mass spectrum of peak at 8.45 min retention time

No	Compound Name	Similarity	Fomula
1	2, 2'-(Ethane-1, 2-diylbis(oxy))bis(ethane-2, 1-diyl) dibenzoate	801	$C_{20}H_{22}O_6$
2	Diethylene glycol dibenzoate	788	$C_{18}H_{18}O_5$
3	1, 3-Dioxolane, 2-(methoxylmethyl)-2-phenyl-	787	$C_{11}H_{14}O_3$
4	Benzoic acid, 2-(3-nitrophenyl)ethyl ester	778	$C_{15}H_{13}NO_4$
5	Benzoic acid, 2-(4-nitrophenoxy)ethyl ester	776	$C_{16}H_{13}NO_5$
6	3, 6, 9, 12-Tetraoxatetradecane-1, 14-diyl dibenzoate	764	$C_{24}H_{30}O_8$
7	Ethanol, 2-(4-phenoxyphenoxy)-, benzoate	752	$C_{21}H_{18}O_4$
8	1, 3-Dioxolane, 2-phenyl-2-(phenylmethyl)-	750	$C_{16}H_{16}O_2$
9	Benzoic acid, 2-(2-chlorophenoxy)ethyl ester	730	$C_{15}H_{13}ClO_3$
10	3, 4-Pyridinedicarboxylic anhydride	703	$C_7H_3NO_3$

Table 3 Results of esimation of elemental composition for the MS peak of 314.11462

Fomula	Calculated *m/z*	Error/mDa
$C_{18}H_{18}O_5$	314.11488	-0.26
$C_{16}H_{16}N_3O_4$	314.11353	1.09
$C_{19}H_{14}N_4O$	314.11621	-1.59
$C_{21}H_{16}NO_2$	314.11756	-2.94
$C_{13}H_{18}N_2O_7$	314.11085	3.77

Table 4 The results of estimation of elemental compositions for the peaks in mass spectrum at 8.45 min peak

m/z of peak	Fomula	Error/mDa
51.02244	C_4H_3	-0.48
77.03809	C_6H_5	-0.49
105.03439	H_7H_5O	0.9
105.08898	$C_5H_{13}O_2$	-2.02
149.05995	$C_9H_9O_2$	0.25

Using this software for library-registered compounds (similarity: high), it is possible to provide highly reliable qualitative analysis results that combine the EI library search results with the SI molecular ion elemental composition results. Furthermore, the msFineAnalysis software made it possible to estimate the molecular ion elemental composition as well as the fragment ion elemental compositions for unknown compounds that do not show a good library match (similarity: low). These results provided a stronger foundation for identifying unknowns than using the conventional EI with library search method alone.

Irrespective of high or low similarity, the new analysis method enables the estimation of molecular composition formulas and to refine candidates for identification. Thus, msFineAnalysis is very effective for qualitative analysis of GC-HRMS (JMS-T200GC) data.

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Introduction of AI Structure Analysis Function in Automatic Structure Analysis Software msFineAnalysis AI

Product used : Mass Spectrometer (MS)

Introduction

Electron ionization (EI) is one of the most popular ionization methods used in gas chromatography-mass spectrometry (GC-MS). Consequently, compounds are typically identified by a mass spectral database search using EI mass spectra. Because molecular ions are often weak or absent in 70 eV EI mass spectra, identification of unknowns can be difficult by EI alone. In these cases, soft ionization (SI) can be very helpful for producing and identifying molecular ions. Recently, JEOL began developing an integrated qualitative analysis workflow that automatically combines and interprets the information from EI and SI data. And then in 2018, we introduced our integrated qualitative analysis software “msFineAnalysis” which uses both EI and SI data to improve compound identification for GC-MS applications.

Despite the fact that msFineAnalysis was automatically able to determine the molecular formula and partial structure information from EI fragment ion formulas, the actual structural formulas still required manual analysis using chemical compositions. To address this, we then developed an automated structure analysis software package entitled “msFineAnalysis AI” which uses artificial intelligence (AI) to predict EI mass spectra from chemical structures. We have used our newly-developed AI model to create a database of predicted EI mass spectra for around 100 million compounds. In this work, we introduce AI structure analysis function in automatic structure analysis software msFineAnalysis AI.

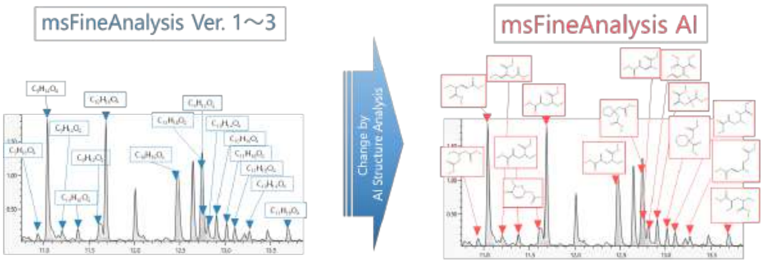

Figure 1 Image of analysis result in msFineAnalysis AI

About AI Structure Analysis Function

AI structure analysis function performs automatic structure the analysis for unknown compounds using two AIs (main AI, support AI) that complementarily combine machine learning and deep learning.

Figure 2 shows the workflow of AI structural analysis by the main AI. In the main AI, a model for EI mass spectra prediction from structural formulas was constructed using deep learning, and predicted EI mass spectra of 100 million compounds were included in the software as an "AI library" database. The database search function using the "AI library" is implemented similarly to traditional library searches using the commercially available EI mass spectra database. Structural formula candidates are narrowed down by molecular formulas uniquely determined by integrated qualitative analysis, so more correct structural formulas can be obtained quickly. The predicted EI mass spectra were compared with measured EI mass spectra, then the scores were calculated from the spectral patterns, and candidate structural formulas were arranged in order of highest score. Finally, the correct structural formula is selected by combining the obtained structural formula candidates with the sample information and the knowledge and know-how obtained from the previous analysis.

Figure 3 shows the workflow of partial structure prediction by the support AI. The support AI assists interpreting analysis results by predicting the partial structure from the measured EI mass spectrum. It is possible to analyze the composition formula of fragment ions and neutral losses obtained from accurate mass analysis and assist in the interpretation of structural information proposed by the main AI.

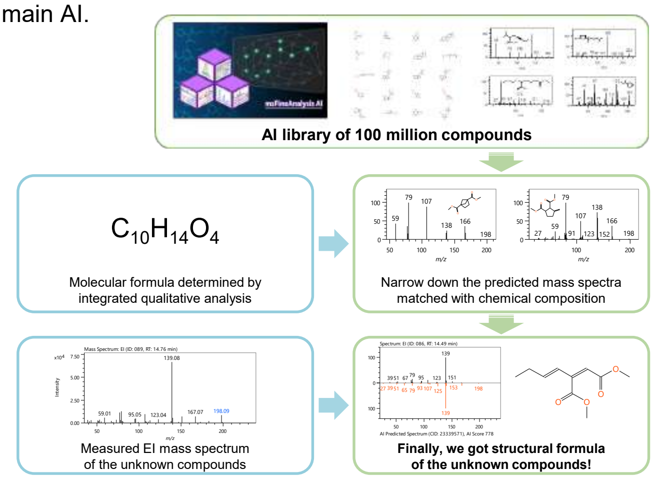
msFineAnalysis AI
AI library of 100 million compounds
$C_{10}H_{14}O_4$
Molecular formula determined by integrated qualitative analysis
Narrow down the predicted mass spectra matched with chemical composition
m/z
Mass Spectrum: EI (ID: 089, RT: 14.76 min)
Intensity
139.08
59.01
95.05
123.04
167.07
198.09
Measured EI mass spectrum of the unknown compounds
Spectrum: EI (ID: 086, RT: 14.49 min)
AI Predicted Spectrum (CID: 23339571), AI Score 778
Finally, we got structural formula of the unknown compounds!

Figure 2 Main AI workflow

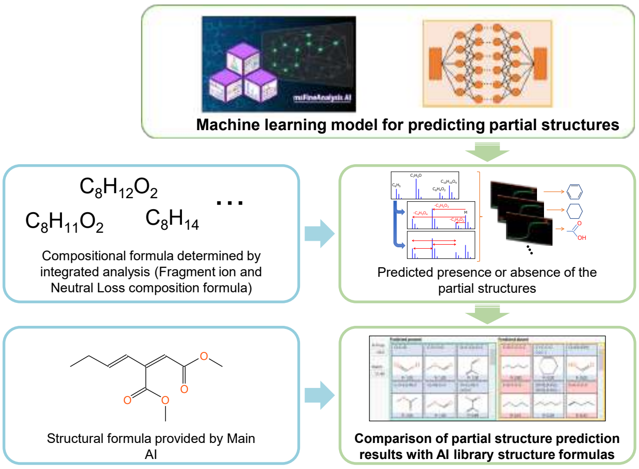
msFineAnalysis AI
Machine learning model for predicting partial structures
$C_8H_{12}O_2$
$C_8H_{11}O_2$
C_8H_{14}
…
Compositional formula determined by integrated analysis (Fragment ion and Neutral Loss composition formula)
Predicted presence or absence of the partial structures
Structural formula provided by Main AI
Comparison of partial structure prediction results with AI library structure formulas

Figure 3 Support AI workflow

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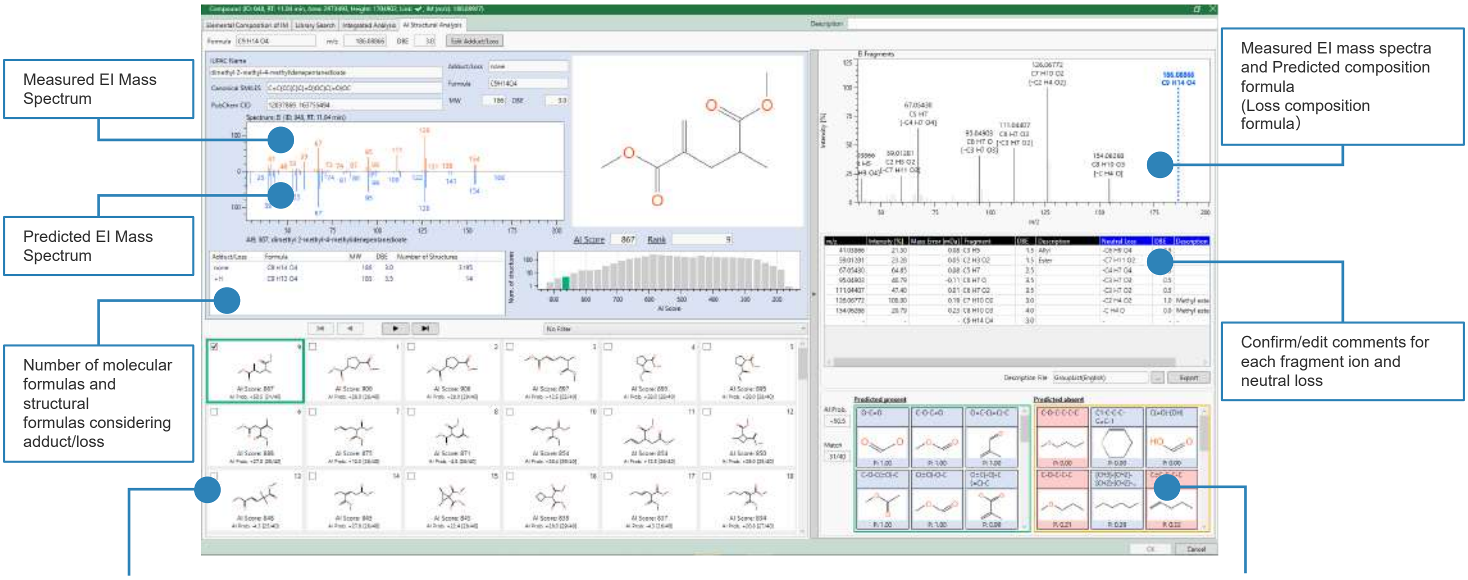
GUI of AI Structure Analysis Result

Figure 4 shows the AI structure analysis result of Acrylic Resin Oligomers by msFineAnalysis AI. The target of analysis is a dimer component that is not registered in the NIST library database. The left side of the analysis result screen shows the structure candidates by the main AI, and the right side shows the analysis results by the support AI. Detailed structural information can be obtained even for unknown compounds that have not been registered in the database.

On the main AI analysis result screen, a list of predicted structural formulas is shown at the bottom of the screen, and it is possible to check the AI structural analysis results all at once. The AI score indicates the similarity between the AI library and the measured mass spectrum , and it is shown at the bottom of each structural formula. Furthermore, information on the selected structural formula is posted at the top of the screen. We can see where the selected structural formula is in the histogram. It also includes a filtering function by partial structure and monomer, which enables structural analysis results to reflect the presence or absence of substructures predicted by the support AI is described below.

On the support AI analysis result screen, predicted partial structure information is shown at the bottom of the screen. On the list, the left side is the partial structure predicted to be present, and the right side is the partial structure predicted not to be present. The partial structure with blue background matches the structural formula selected in the main AI, while the partial structure with red background does not match. Measured mass spectrum and the predicted composition formula of each fragment ion/neutral loss is posted at the top of the screen. It is also possible to confirm and edit comments for each estimated composition formula.


Measured EI Mass Spectrum
Predicted EI Mass Spectrum
Number of molecular formulas and structural formulas considering adduct/loss
Measured EI mass spectra and Predicted composition formula (Loss composition formula)
Confirm/edit comments for each fragment ion and neutral loss

Structural Formula Prediction by Main AI

- Displays ranked structural formulas in list.
- Selecting a structural formula updates the information that is displayed.
- Below each structural formula is an AI score that indicates the match percentage between structural formula and mass spectrum.

Partial Structure Prediction by Support AI

- Displays the predicted partial structure information
- Partial structure predicted and present are on the left, predicted and absent are on the right.
- Those with a blue background are the partial structures that match the selected structural formulas. Those with a red background are those that do not match.

Figure 4 GUI of msFineAnalysis AI

Conclusion

In this MSTips, we introduced our newly-developed software msFineAnalysis AI, which contains AI structural analysis functionality to enhance qualitative analysis workflow. This software performs automatic structure analysis for unknown compounds using two AIs (main AI, support AI) that complementarily combine machine learning and deep learning. No knowledge of mass spectrometry and AI are required as the software automatically interprets complex mass spectra.

Qualitative analysis of GC-MS data can be greatly assisted by using EI and SI data together with msFineAnalysis AI, especially when trying to identify unknown compounds in complex samples.

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JMS-T2000GC AccuTOF™ GC-Alpha
Sensitivity in nitrogen carrier gas ① - EI / PI ion source

Related products: Mass spectrometer (MS)

Introduction

Due to the global shortage of helium gas supply, the demand for alternative gas for GC-MS carrier gas is increasing. Nitrogen gas is the most suitable gas due to its availability and high safety, but it is known that the influence of nitrogen ions generated by the MS ion source causes a decrease in sensitivity. So we have checked the influences of nitrogen carrier gas on JMS-T2000 GC AccuTOF ™ GC-Alpha, and report on MS Tips No. 374-376. This report shows the results of the EI (Electron Ionization) / PI (Photo Ionization) combination ion source, which is one of the characteristic multi-ionization ion sources of JMS-T2000 GC AccuTOF ™ GC-Alpha.

Measurement

Table 1 shows the details of the measurement conditions in this experiment. In the EI method, 1 µL of OFN (octafluoronaphthalene) 100 pg / µL was injected. In the PI method, 1 µL of benzophenone 10 ng / µL was injected. Helium and nitrogen were used as carrier gases, and the S/N sensitivity, the similarity to the library spectrum (M.F.), and the mass accuracy (error) of molecular ions were compared. The carrier gas flow rate was set to 1.0 mL / min in helium and 0.55 mL / min in nitrogen based on the optimum linear velocity of each carrier gas. The ionization energy in the EI method was measured at 70eV and 20 eV, which is expected to suppress the ionization of nitrogen.

Table 1. Measurement conditions

GC : 8890GC (Agilent Technologies, Inc.)	
Injection volume	1 µL
Mode	Splitless
Column	DB-5MS UI (Agilent Technologies, Inc.) 30m x 0.25mm, 0.25µm
Oven temperature	40°C(1min)-30°C/min -250°C(2min)
Carrier flow	He : 1.0 mL/min N_2 : 0.55 mL/min

TOFMS : JMS-T2000GC AccuTOF™ GC-Alpha	
Ion source	EI/PI combination ion source
Ionization	①EI, ②PI
EI Ionization energy (filament current)	70eV (300µA), 20eV (200µA)
Mass Range	*m/z* 35-600
Detector voltage	①2600V, ②2800V

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Results ① EI method

Figure 1 shows the extracted ion chromatograms (*m/z* 272.98 ± 0.10) of the OFN measurement results in the EI method. The sensitivity was greatly decreased to about 1/30 in nitrogen (70 eV). In the nitrogen (20 eV), the sensitivity was slightly decreased to about 1/3. It was confirmed that the decrease in sensitivity was suppressed by changing the ionization energy.

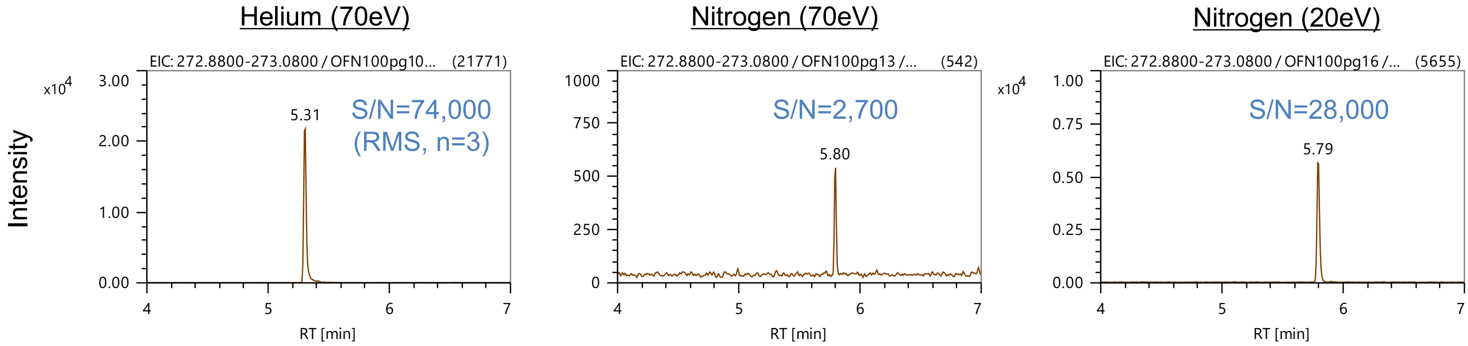

Figure 1. EICs of OFN（EI method)

Figure 2 shows the mass spectra of the OFN measurement results in the EI method. The similarities to the library spectra (M.F.) were good at 800 or more in helium (70eV) and nitrogen (70eV). It was slightly decreased to about 760 in nitrogen (20eV), since the low energy ionization suppressed the fragments and changed the spectrum. The mass errors of the molecular ions $M^{+\cdot}$ (*m/z* 271.9867) were as good as 1 mDa or less in all results.

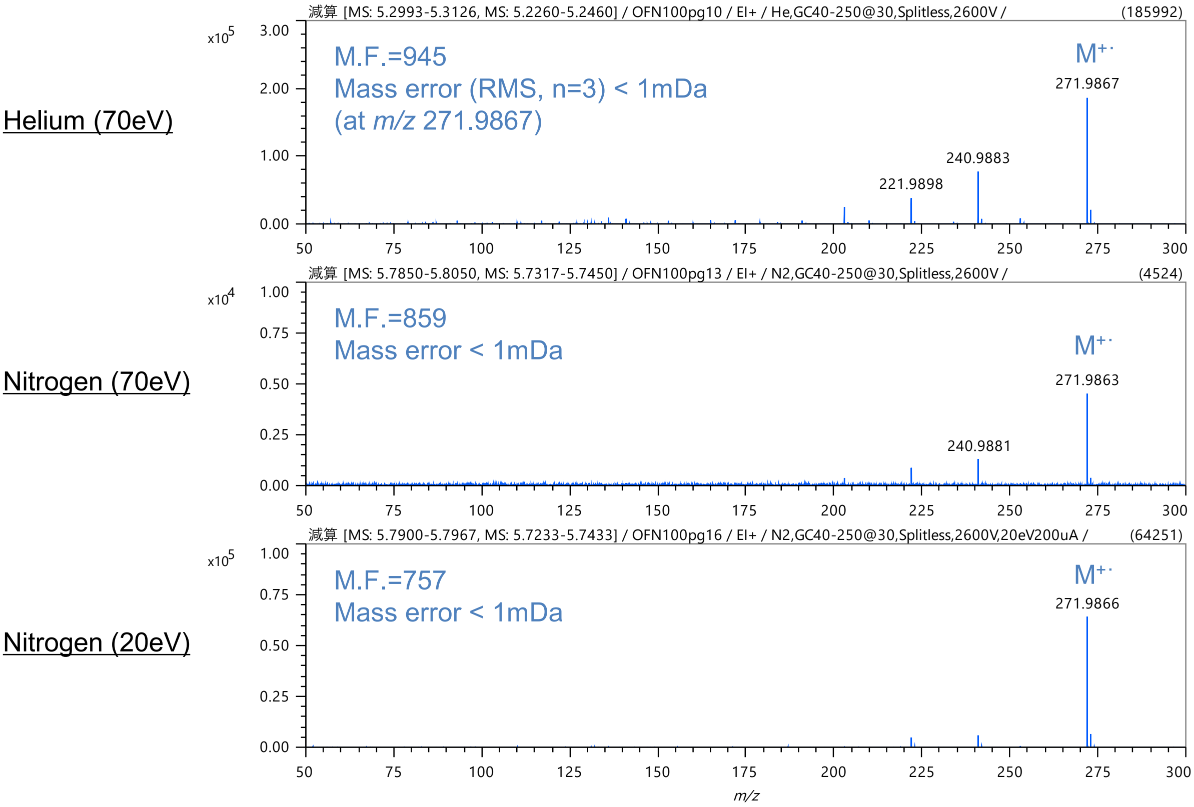

Figure 2. Mass spectra of OFN（EI method)

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Results ② PI method

Figure 3 shows the extracted ion chromatograms (*m/z* 182.07 ± 0.10) of the benzophenone in the PI method. The sensitivity was slightly decreased to about 1/3. In the PI method, which is soft ionization, nitrogen is hardly ionized, but the sensitivity is slightly reduced due to the influence of a large amount of nitrogen molecules.

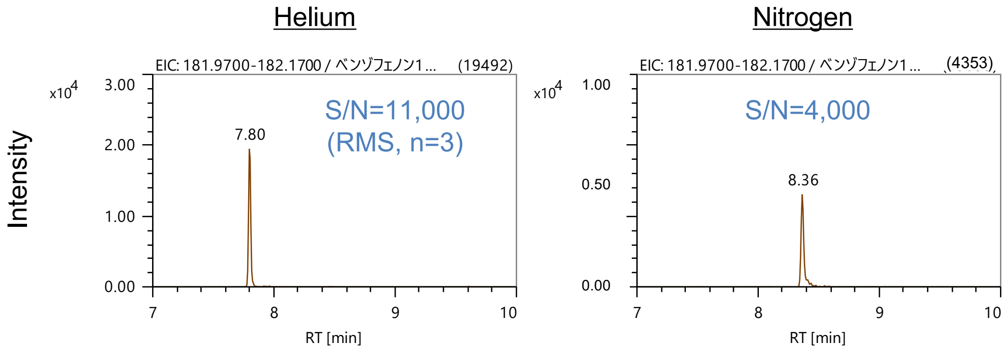

Figure 3. EICs of benzophenone（PI method)

Figure 4 shows the mass spectra of the benzophenone in the PI method. The mass errors of the molecular ions $M^{+\cdot}$ (*m/z* 182.0726) were as good as 1 mDa or less in both results.

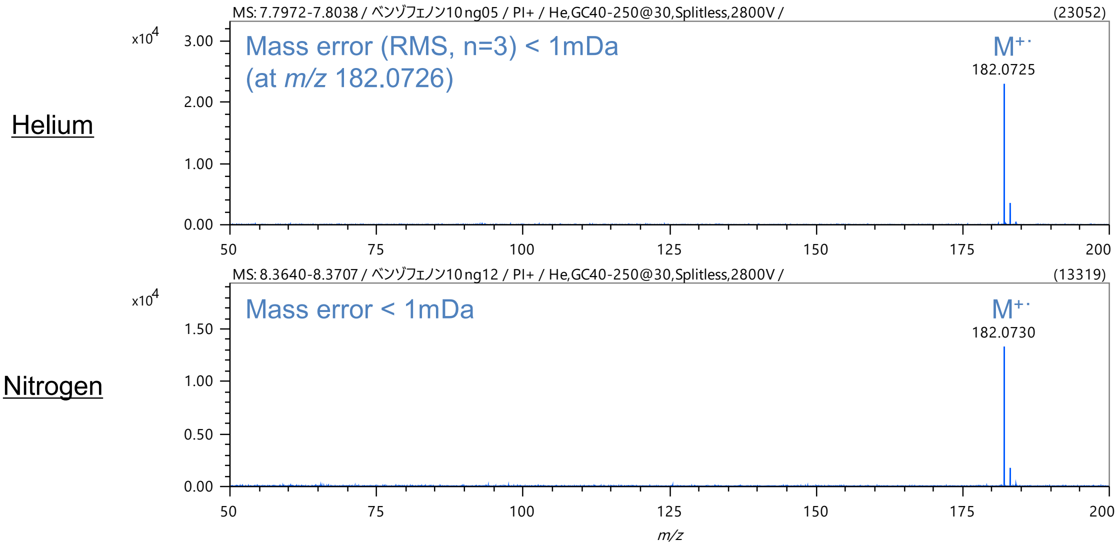

Figure 4. Mass spectra of benzophenone（PI method)

Conclusion

The influences of nitrogen carrier on the EI / PI combination ion source of JMS-T2000GC AccuTOF ™ GC-Alpha were checked. In the EI method, the sensitivity was greatly decreased to about 1/30, but it could be suppressed by changing the ionization energy. In the PI method, the sensitivity was slightly decreased to about 1/3. The mass errors of the molecular ions were as good as 1 mDa or less in both EI method and PI method.

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JMS-T2000GC AccuTOF™ GC-Alpha
Sensitivity in nitrogen carrier gas ② - EI / FI ion source

Related products: Mass spectrometer (MS)

Introduction

Due to the global shortage of helium gas supply, the demand for alternative gas for GC-MS carrier gas is increasing. Nitrogen gas is the most suitable gas due to its availability and high safety, but it is known that the influence of nitrogen ions generated by the MS ion source causes a decrease in sensitivity. So we have checked the influences of nitrogen carrier gas on JMS-T2000 GC AccuTOF ™ GC-Alpha, and report on MS Tips No. 374-376. This report shows the results of the EI (Electron Ionization) / FI (Field Ionization)combination ion source, which is one of the characteristic multi-ionization ion sources of JMS-T2000 GC AccuTOF ™ GC-Alpha.

Measurement

Table 1 shows the details of the measurement conditions in this experiment. In the EI method, 1 μL of OFN (octafluoronaphthalene) 100 pg / μL was injected. In the FI method, 1 μL of hexadecane 10 ng / μL was injected. Helium and nitrogen were used as carrier gases, and the S/N sensitivity, similarity to the library spectrum (M.F.), and mass accuracy (error) of molecular ions were compared. The carrier gas flow rate was set to 1.0 mL / min in helium and 0.55 mL / min in nitrogen based on the optimum linear velocity of each carrier gas. The ionization energy in the EI method was measured at 20 eV, which is expected to suppress the ionization of nitrogen, in addition to the general 70 eV.

Table 1. Measurement conditions

GC : 8890GC (Agilent Technologies, Inc.)	
Injection volume	1 μL
Mode	Splitless
Column	DB-5MS UI (Agilent Technologies, Inc.) 30m x 0.25mm, 0.25μm
Oven temperature	40°C(1min)-30°C/min -250°C(2min)
Carrier flow	He : 1.0 mL/min N_2 : 0.55 mL/min

TOFMS : JMS-T2000GC AccuTOF™ GC-Alpha	
Ion source	EI/FI combination ion source
Ionization	①EI, ②FI
EI Ionization energy (filament current)	70eV (300μA), 20eV (200μA)
Mass Range	*m/z* 35-600
Detector voltage	2600V

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Results ① EI method

Figure 1 shows the extracted ion chromatograms (*m/z* 272.98 ± 0.10) of the OFN measurement results in the EI method. The sensitivity was decreased about 1/3 in nitrogen (70 eV). Since the EI / FI shared ion source has an open structure without a chamber, nitrogen retention in the ion source is small. Therefore, it is considered that the influence of nitrogen ions was small and the sensitivity decrease was suppressed. In nitrogen (20eV), which was expected to suppress the sensitivity decrease, the sensitivity was further decreased. It was confirmed that it is not necessary to change the ionization energy in the EI / FI ion source.

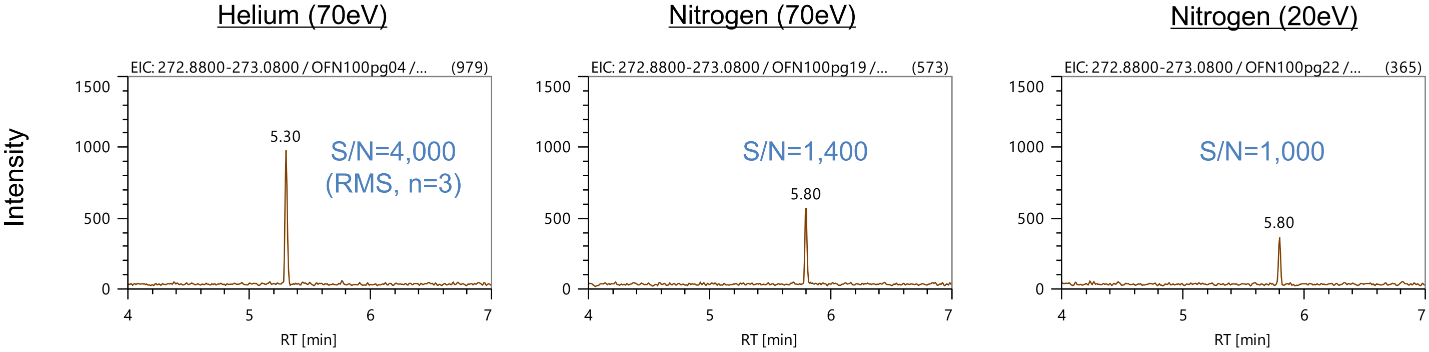

Figure 1. EICs of OFN（EI method)

Figure 2 shows the mass spectra of the OFN measurement results in the EI method. The similarities to the library spectra (M.F.) were good at 800 or more in helium (70eV) and nitrogen (70eV). It was decreased to about 590 in nitrogen (20eV)), since the low energy ionization suppressed the fragments and changed the spectrum. The mass error of the molecular ion $M^{+\cdot}$ (*m/z* 271.9867) was 1 mDa or less in helium (70 eV). They were decreased to 2 mDa or less in nitrogen (70 eV) and nitrogen (20 eV).

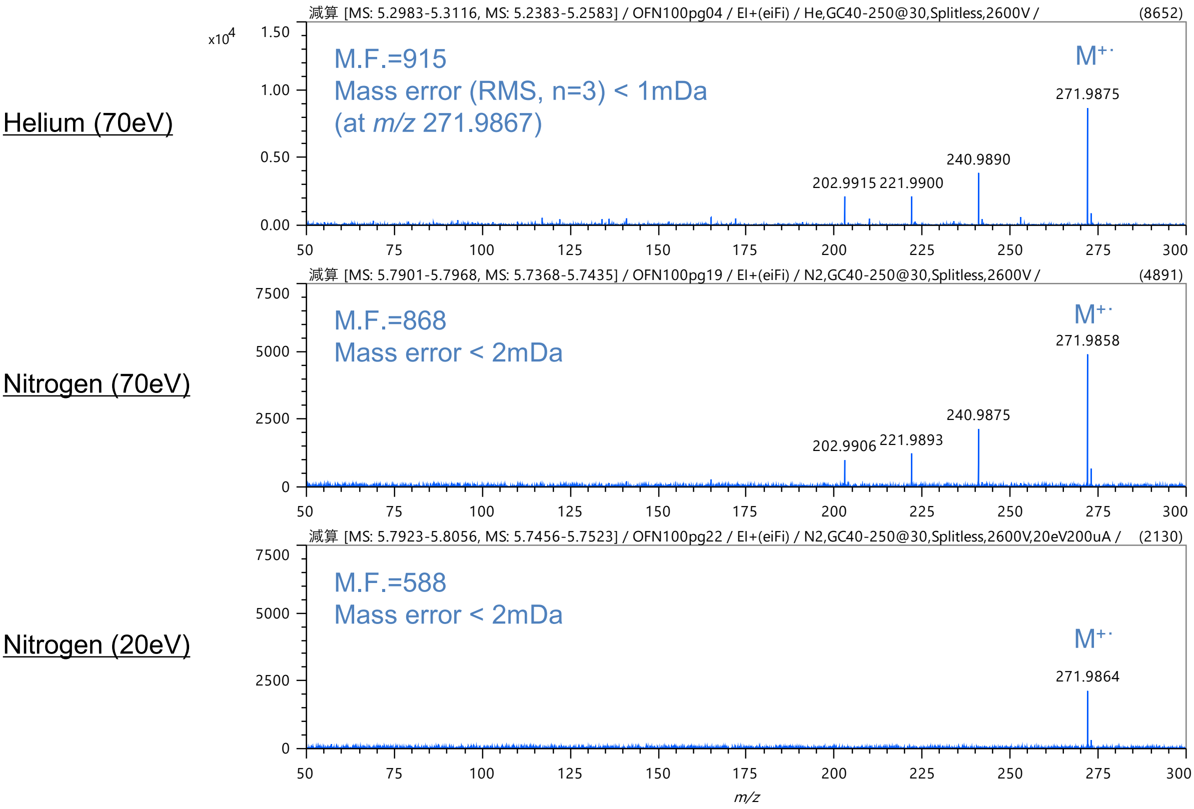

Figure 2. Mass spectra of OFN（EI method)

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Results ② FI method

Figure 3 shows the extracted ion chromatograms (m/z 226.26 ± 0.10) of the hexadecane measurement result in the FI method. The sensitivity was almost the same in helium and nitrogen. Since nitrogen is hardly ionize in the FI method, which is soft ionization, the decrease in sensitivity was suppressed.

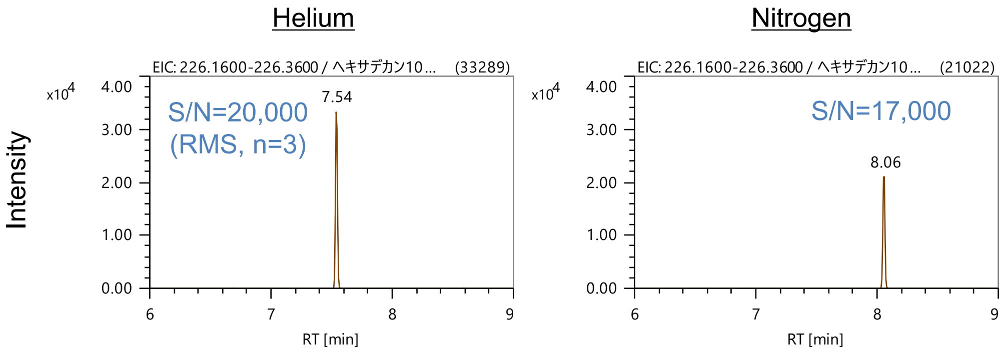

Figure 3. EICs of hexadecane（FI method)

Figure 4 shows the mass spectra of the hexadecane measurement results in the FI method. The mass error of the molecular ions $M^{+\cdot}$ (m/z 226.2655) were 2 mDa or less in both results.

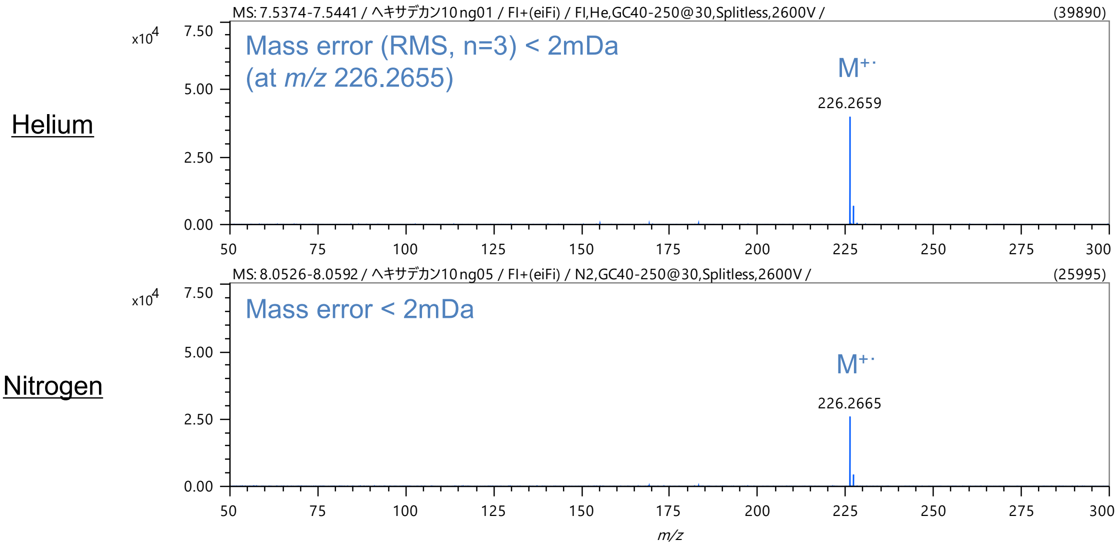

Figure 4. Mass spectra of hexadecane（FI method)

Conclusion

The influences of nitrogen carriers on the EI / FI combination ion source of JMS-T2000GC AccuTOF ™ GC-Alpha were checked. In the EI method, the sensitivity was decreased to about 1/3. In the FI method, the sensitivity was not decreased. The mass errors of the molecular ions were as good as 2 mDa or less in both EI method and FI method.

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Applications note

MS
MSTips No. 376
GC-TOFMS Application

JMS-T2000GC AccuTOF™ GC-Alpha Sensitivity in nitrogen carrier gas ③ - CI ion source

Related products: Mass spectrometer (MS)

Introduction

Due to the global shortage of helium gas supply, the demand for alternative gas for GC-MS carrier gas is increasing. Nitrogen gas is the most suitable gas due to its availability and high safety, but it is known that the influence of nitrogen ions generated by the MS ion source causes a decrease in sensitivity. So we have checked the influences of nitrogen carrier gas on JMS-T2000 GC AccuTOF ™ GC-Alpha, and report on MS Tips No. 374-376. This report shows the results of the CI (Chemical Ionization) ion source.

Measurement

Table 1 shows the details of the measurement conditions in this experiment. In the positive ion CI (CI+) method, 1 μL of benzophenone 100 pg / μL was injected. In the negative CI (CI-) method, 1 μL of OFN (octafluoronaphthalene) 10 pg / μL were injected. Helium and nitrogen were used as carrier gases, and the S/N sensitivity and mass accuracy (error) of molecular ions were compared. The carrier gas flow rate was set to 1.0 mL / min in helium and 0.6 mL / min in nitrogen based on the optimum linear velocity of each carrier gas.

Table 1. Measurement conditions

GC : 8890GC (Agilent Technologies, Inc.)	
Injection volume	1 μL
Mode	Splitless
Column	DB-5MS UI (Agilent Technologies, Inc.) 30m x 0.25mm, 0.25μm
Oven temperature	50°C(1min)-40°C/min -250°C(2min)
Carrier flow	He : 1.0 mL/min N_2 : 0.6 mL/min

TOFMS : JMS-T2000GC AccuTOF^{TM} GC-Alpha	
Ion source	CI ion source
Ionization	①CI+, ②CI-
CI reaction gas	Methane
Ionization energy (filament current)	200eV (300μA)
Mass Range	*m/z* 100-500
Detector voltage	2500V

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Results ① CI+ method

Figure 1 shows the extracted ion chromatograms (*m/z* 183.08 ± 0.02) of the measurement result of benzophenone in the CI+ method. The sensitivity was decreased to about 1/2 in nitrogen. Since nitrogen is difficult to ionize in the CI method, which is soft ionization, the decrease in sensitivity was suppressed.

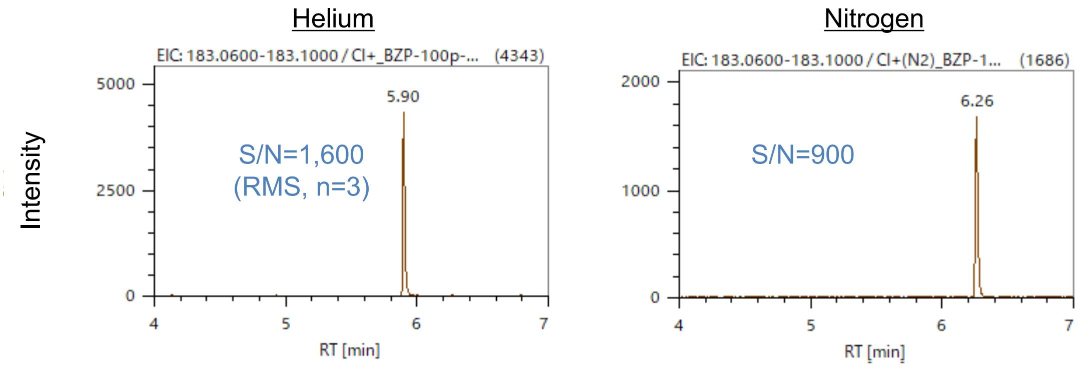

Figure 1. EICs of benzophenone（CI+ method)

Figure 2 shows the mass spectra of the benzophenone measurement result in the CI+ method. Protonated ions [M+H]$^+$ (*m/z* 183.0804) were strongly observed, and their mass errors were as good as 1 mDa or less in both results.

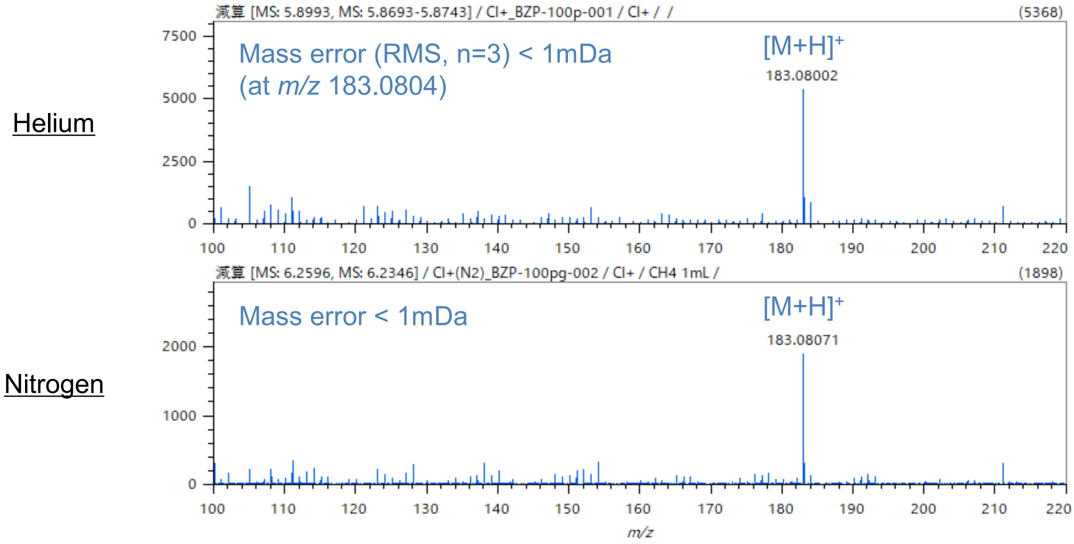

Figure 2. Mass spectra of benzophenone（CI+ method)

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Results ②CI- method

Figure 3 shows the extracted ion chromatograms (*m/z* 271.99 ± 0.02) of the OFN measurement result in the CI- method. The sensitivity was improved about twice in nitrogen. Since it was difficult to ionize reaction gas impurity in addition to nitrogen in CI- method, which is soft ionization, it is considered that the sensitivity was improved.

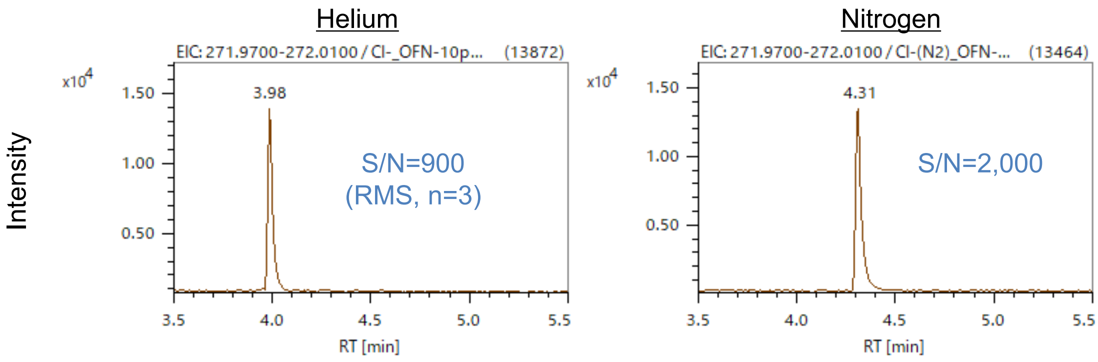

Figure 3. EICs of OFN（CI- method)

Figure 4 shows the mass spectra of the OFN measurement result in the CI- method. The mass errors of the molecular ions $M^{-\cdot}$(*m/z* 271.9878) were as good as 1 mDa or less in both results.

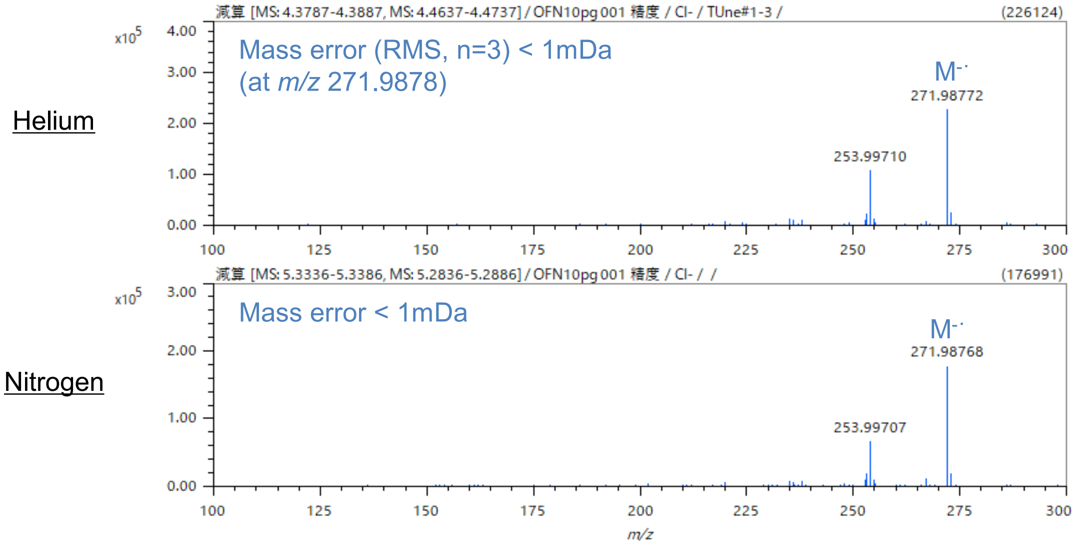

Figure 4. Mass spectra of OFN（CI- method)

Conclusion

The influences of nitrogen carriers on the CI ion source of JMS-T2000GC AccuTOF ™ GC-Alpha were checked. In the CI+ method, the sensitivity was decreased to about 1/2. In the CI- method, the sensitivity was not decreased. The mass errors of the molecular ions were as good as 1 mDa or less in both CI+ method and CI- method.

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Effect of JMS-T2000GC high mass resolution on the analysis result —KMD Plot comparison using msRepeatFinder—

Related Product: Mass Spectrometer(MS)

Introduction

Recently, JEOL announced the release of the JMS-T2000GC “AccuTOF™ GC-Alpha”, which is the 6th generation GC-HRTOFMS in the JEOL “AccuTOF™ GC” series (Fig. 1). The GC-Alpha achieves three times higher mass resolving power (10,000→30,000 @ *m/z* 614) and three times higher mass accuracy (3ppm→1ppm, EI standard ion source) than the previous model. In this work, we used direct probe field desorption (FD) of crude oil (a very complex mixture) to monitor the effects of improved resolution. Additionally, the JEOL msRepeatFinder software was used to examine the crude oil data by using Kendrick mass defect (KMD) plots in order to more clearly visualize the effects of improved mass resolution on the analysis results.

Fig. 1 JEOL GC-HRTOFMS systems: JMS-T2000GC

Experimental

Crude oil from the Gulf of Mexico (SRM2779, NIST) was used as the sample. The previous generation JMS-T200GC and new generation JMS-T2000GC equipped with EI/FI/FD combination ion sources were used for the sample analysis. Table 1 shows the measurement conditions for these systems. The data from each system was then analyzed by using msRepeatFinder to confirm the effect of improved mass-resolving power on the analysis results.

Table 1. Measurement and analysis conditions

MS conditions	
Spectrometer	JMS-T2000GC (JEOL Ltd.) JMS-T200GC (JEOL Ltd.) (Previous model)
Ion Source	EI/FI/FD combination ion source
Ionization	FD+: -10kV, 0→51.2mA/min→50mA
Mass Range	*m/z* 35-1,600
Data processing condition	
Software	msRepeatFinder (JEOL Ltd.)

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Result

Fig. 2 shows the FD mass spectrum obtained by both instruments. Although the overall spectrum patterns were very similar, a closer inspection of the peaks showed that the peak separation differed significantly for each instrument. The JMS-T2000GC (Fig. 2(a)) showed a clear mass separation of each hydrocarbon component, even in the high mass range above m/z 600, that is the result of the new system having a higher mass resolving power. In contrast, the mass separation for the previous model was insufficient, particularly in the high mass range, to adequately resolve the mass peaks from each other.

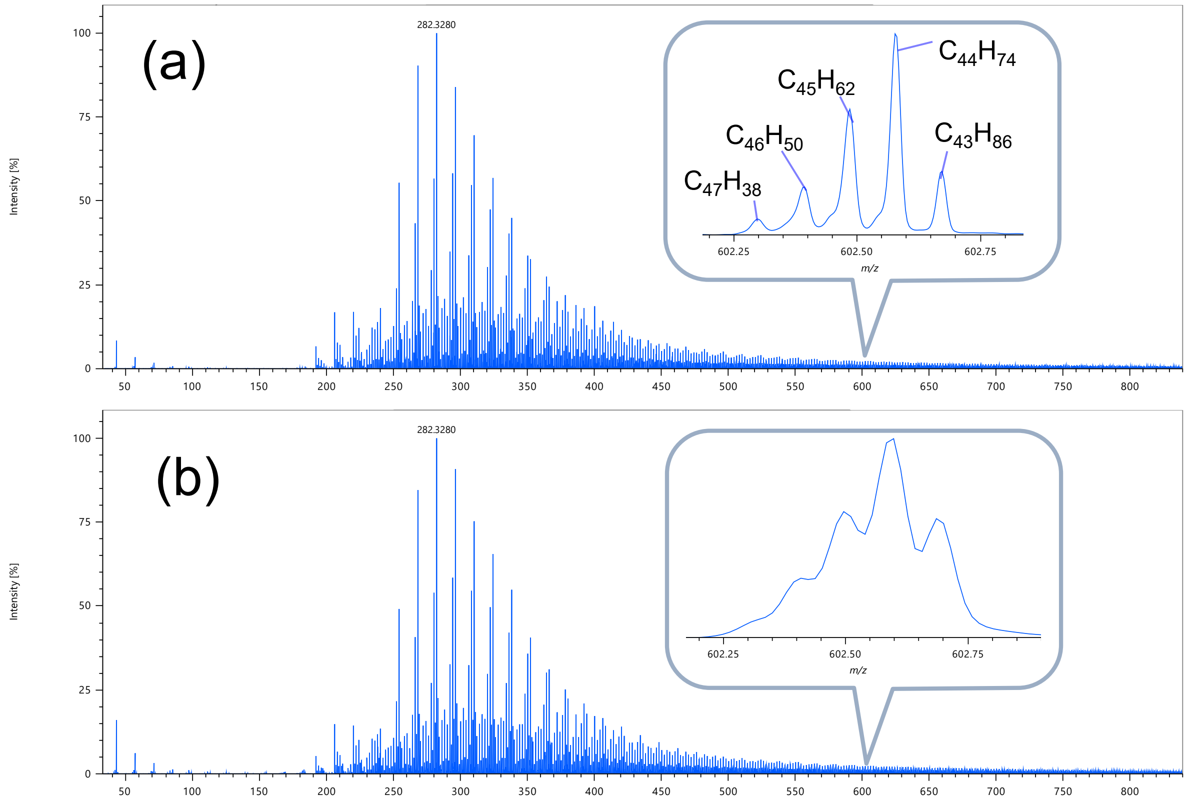

Fig. 2 FD mass spectra for crude oil: (a) JMS-T2000GC data, (b) Previous model data

Next, msRepeatFinder was used to visualize each FD mass spectrum with KMD plots (Fig. 3). The JMS-T2000GC KMD plot (Fig. 3(a)) clearly showed the family of components over the full mass range (including the components above *m/z* 600). This outcome is a direct result of the improved peak separation, as shown in Fig. 2. On the other hand, the KMD plot for the previous model showed very poor results for the peaks above m/z 600, with many components disappearing because of insufficient mass separation. Basically, the unresolved peaks are being treated as single components despite the fact that there are multiple components, thus resulting in the loss of KMD Plot information in the high mass range.

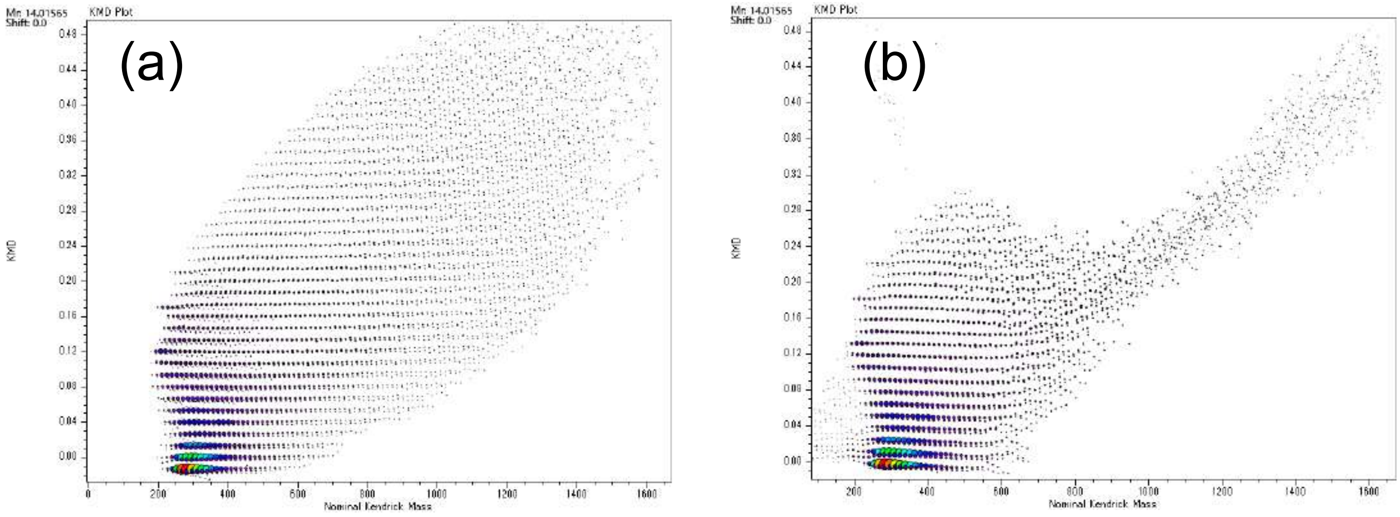

Fig. 3 KMD Plots of FD mass spectrum: (a) JMS-T2000GC data, (b) Previous model data

Conclusions

The above results confirmed that the high resolving power achieved by the JMS-T2000GC was particularly effective for direct mass measurements (FD probe) of complex materials like crude oil. Additionally, the KMD plots clearly showed that the T2000GC high mass-resolution dramatically increased the number of detected components as a result of the improved mass separation.

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Applications note

MS
MSTips : 331
GC-TOFMS Application

Effect of high mass accuracy on the analysis result by JMS-T2000GC－Effect to narrow down the result of msFineAnalysis integrated analysis－

Related Product: Mass Spectrometer(MS)

Introduction

JEOL Ltd. recently announced the JMS-T2000GC “AccuTOF^{TM} GC-Alpha” which is the 6th generation GC high resolution time-of-flight MS (GC-HRTOFMS) in the “AccuTOF^{TM} GC” series that was first released in 2004. The GC-Alpha (Fig. 1) represents a significant improvement in capabilities over the previous model with three times higher mass resolving power (10,000→30,000 @ *m/z* 614) and three times higher mass accuracy (3ppm→1ppm, EI standard ion source). In this work, we used the thermal decomposition of an acrylic resin to evaluate how improved mass accuracy can affect the analysis results for a complex sample. Additionally, the msFineAnalysis Version 3 software included with the JMS-T2000GC was used to quickly determine the impact of improved mass accuracy on the qualitative analysis results.

Experimental

Table 1 shows the measurement conditions for the pyrolysis GC-MS measurements. A JMS-T2000GC equipped with a Frontier Lab pyrolyzer and the JEOL EI/FI combination ion source was used for the measurements, and a commercially available acrylic resin was used as the sample -- 0.2mg for EI method and 1.0mg for FI method, respectively. The resulting data was then analyzed by using the msFineAnalysis integrated workflow (next section, Fig. 2) to examine the effects of high mass accuracy on the analysis results.

Table 1. Measurement and analysis conditions

Pyrolysis conditions	
Pyrolyzer	EGA/PY-3030D(Frontier Lab)
Pyrolysis Temperature	600ºC
GC conditions	
Gas Chromatograph	8890A GC
	(Agilent Technologies)
Column	ZB-5MSi (Phenomenex)
	30m x 0.25mm, 0.25µm
Oven Temperature	40ºC(2min)-10ºC/min
	-320ºC(15min)
Injection Mode	Split mode (100:1)
Carrier flow	He:1.0mL/min

MS conditions	
Spectrometer	JMS-T2000GC (JEOL Ltd.)
Ion Source	EI/FI combination ion source
Ionization	EI+:70eV, 300µA
	FI+:-10kV, 40mA/30msec
Mass Range	*m/z* 35-800
Data processing condition	
Software	msFineAnalysis (JEOL Ltd.)
Library database	NIST17
Tolerance	±5mDa、±2mDa

Fig. 1 JEOL GC/HR-TOFMS systems: JMS-T2000GC

msFineAnalysis Software

Fig. 2 shows the msFineAnalysis workflow in which GC/EI data and GC/soft ionization (SI) data are analyzed together to automatically produce an integrated qualitative analysis report. The 5 qualitative analysis steps that are automatically executed are:

1. Library database search using EI mass spectrum
2. Automatic search of molecular ion in the SI mass spectrum
3. Accurate mass analysis for the molecular ion
4. Isotope pattern matching analysis to narrow down the candidate molecular formulas
5. Accurate mass analysis of EI fragment ion and narrowing down molecular formula candidates by using the composition condition of molecular formula candidate obtained in 1 and 4.

By combining the accurate mass analysis of the EI and SI mass spectra, msFineAnalysis cannot only identify components registered in the library but can also determine the elemental composition for unregistered components.

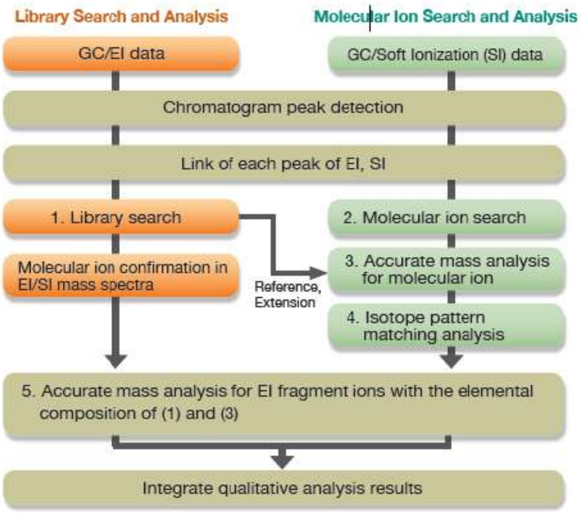
Library Search and Analysis
Molecular Ion Search and Analysis
GC/EI data
GC/Soft Ionization (SI) data
Chromatogram peak detection
Link of each peak of EI, SI
1. Library search
2. Molecular ion search
Molecular ion confirmation in EI/SI mass spectra
Reference, Extension
3. Accurate mass analysis for molecular ion
4. Isotope pattern matching analysis
5. Accurate mass analysis for EI fragment ions with the elemental composition of (1) and (3)
Integrate qualitative analysis results

Fig. 2 msFineAnalysis workflow

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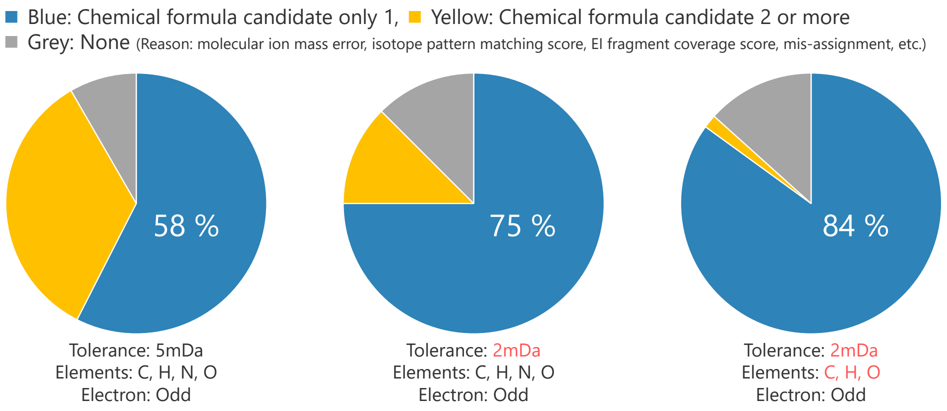
Result

For accurate mass analysis, an error tolerance is specified based on the mass accuracy capabilities of the instrument. The previous generation models (i.e. JMS-T200GC) required an error tolerance of ±5 mDa for the elemental compositions. However, with the higher mass accuracy of the JMS-T2000GC, it is possible to narrow this error tolerance, which in turn lowers the number of possible elemental compositions calculated for each analyte. The goal of these experiments was to examine the effect of error tolerance for the 120 components (Intensity ≥0.05%) that were observed during thermal decomposition of the acrylic resin.

Fig. 3 shows the results for the automatic analysis for the pyrolysis of acrylic resin using msFineAnalysis. The blue color shows the percentage of components that resulted in one molecular formula candidate, the yellow shows the percentage that had two or more molecular formula candidates, and the gray shows the percentage with no clear molecular formula candidate. The left pie chart shows the analysis results when using an error tolerance of ±5 mDa. Because of this wider tolerance, there were many analytes with more than one candidate molecular formula (yellow). Consequently, only 58% of the 120 components were narrowed down to a single molecular formula candidate. Next, the error tolerance was lowered to±2 mDa, and the results are shown in the Fig.2 central pie chart. The narrower tolerance eliminated many false positive candidates and increased the number of components with only one molecular formula candidate to 75%. Next, the elements used for the elemental composition calculations were narrowed to include only C/H/O because the acrylic resin substructure only includes these elements. The pie chart on the right shows the results of removing nitrogen from the search while continuing to use the narrower tolerance of ±2mDa. As a result, the number of components identified with one molecular formula increased to 84% (101 components).



Tolerance: 5mDa
Elements: C, H, N, O
Electron: Odd

Tolerance: 2mDa
Elements: C, H, N, O
Electron: Odd

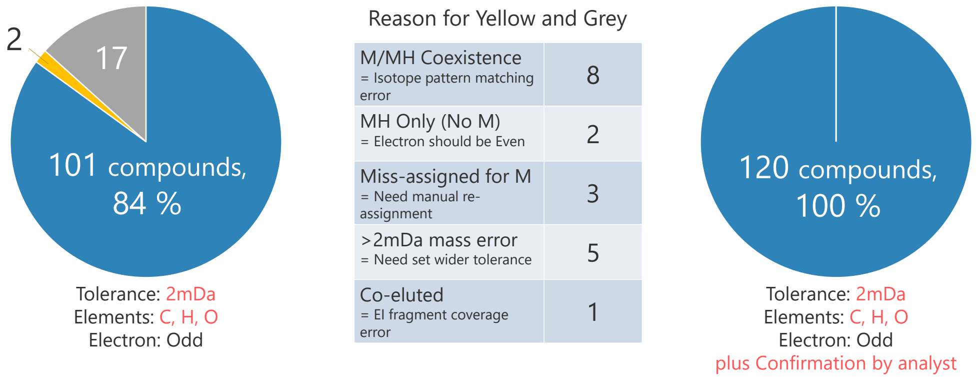
Tolerance: 2mDa
Elements: C, H, O
Electron: Odd

Fig. 3 Comparison of automatic analysis results for 120 components

The remaining 19 components were not automatically narrowed down to a single candidate composition for the following reasons:

- [M]+· and $[M+H]^{+}$ were present together so the isotope pattern did not match.
- Only $[M+H]^{+}$ were observed (since the number of electrons in the proton-added molecule is even, the Odd electron search constraint did not give the correct result).
- The relative intensity of the molecular ion was lower than the default threshold of 10% for ion peak detection and was not correctly assigned.
- The absolute intensity of the molecular ion was low and the peak shape was poor, resulting in a mass error of more than 2mDa.
- It was considered to be a fully co-eluting component, and the EI fragment ion coverage was low.

By manually verifying the measurement data and analysis results for these final 19 components, we were able to narrow down the list to one candidate molecular formula (Fig. 4), thus identifying a single elemental composition for all 120 components that resulted from the pyrolysis of acrylic resin.



Reason for Yellow and Grey

M/MH Coexistence = Isotope pattern matching error	8
MH Only (No M) = Electron should be Even	2
Miss-assigned for M = Need manual re-assignment	3
>2mDa mass error = Need set wider tolerance	5
Co-eluted = EI fragment coverage error	1

Tolerance: 2mDa
Elements: C, H, O
Electron: Odd

Tolerance: 2mDa
Elements: C, H, O
Electron: Odd
plus Confirmation by analyst

Fig. 4 Confirmation by analyst for 19 components

Conclusions

The high mass accuracy of the new JMS-T2000GC allows the analyst to use narrower mass error tolerances within msFineAnalysis. As a result, the software was able to automatically narrow down the number of molecular formula candidates to a single possibility for the majority of the observed components. For components that had more than one candidate formula (yellow) or did not have a formula candidate (gray), the analyst was able to quickly focus on these components and manually verify the mass spectrum and analysis results. The combination of the JMS-T2000GC with the automatic analysis capabilities of the msFineAnalysis software provides a powerful solution that simplifies the qualitative analysis of complex samples.

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GC nitrogen carrier gas solution: Comparing helium and nitrogen in GC-TOFMS exact mass analysis

Product: Mass spectrometer (MS)

Introduction

The global shortage of helium (He), which is used as a GC carrier gas, is becoming a serious issue for various research organizations. As replacement carrier gases such as hydrogen (H_2) and nitrogen (N_2) are being tried, nitrogen, which is inexpensive and safe, is attracting increased attention. However, nitrogen as a GC carrier gas is known to compromise peak separation and sensitivity of chromatograms as well as qualitative analysis accuracy due to changes in mass spectral data, collectively affecting the analytical results.

In this work, we examined the effects of nitrogen carrier gas on the performance of our GC-TOFMS for high resolution, accurate mass analysis. As a model application, an acrylic resin, which was introduced in MS Tips 300, was subjected to pyrolysis analysis. EI and FI were used for ionization. Resulting exact mass data was analyzed by msFineAnalysis to derive estimated molecular formulas. The above measurement was performed with helium and nitrogen carriers, and resulting chromatograms and exact mass data were compared to examine the effect of the carrier gases. The EI/FI ion source used for the measurement has an advantage that it does not affect sensitivity when nitrogen is used as a carrier, as discussed in MS Tips 299. Therefore, it was expected that the analytical results with nitrogen carrier gas would be comparable to those with helium carrier gas.

Experiment

A commercial acrylic resin was used in the same manner as described in MS Tips 300; 0.2 mg for EI and 1.0 mg for FI. Measurement conditions for pyrolysis-GCTOFMS were based on those in MS Tips 300 except that the column flow rate was changed to 1.0 mL/min for helium and 0.55 mL/min for nitrogen according to their respective optimum linear velocity. Table 1 shows the measurement conditions.

In the acquired results, peak separation and sensitivity (intensity and S/N) were compared in the total ion current chromatograms (TICC) and extracted ion chromatograms (EIC) for representative compounds. Using the msFineAnalysis integrated analysis, the estimated molecular formulas were derived for the top 100 most intense peaks. For fairness, auto analysis results were compared without data processing. Factors such as mass accuracy, isotopic pattern matching, and EI fragment ion cover ratio, in addition to peak separation and sensitivity of chromatograms, affect the determination of estimated compounds, thus making comprehensive evaluation of the exact mass analysis possible.

Table 1. Measurement Conditions

Pyrolysis Conditions	
Pyrolyzer	EGA/PY-3030D (Frontier Lab)
Pyrolysis Temperature	600°C
GC conditions	
Gas Chromatograph	7890A GC
	(Agilent Technologies)
Column	ZB-5MSi (Phenomenex)
	30 m x 0.25 mm, 0.25 mm
Oven Temperature	40°C (2 min) - 10°C/min
	-320°C (15 min)
Injection Mode	Split mode (100:1)
Carrier flow	He: 1.0mL/min
	N_2: 0.55mL/min

MS Conditions	
Spectrometer	JMS-T200GC (JEOL Ltd.)
Ion Source	EI/FI combined ion source
Ionization	EI+:70eV, 300μA
	FI+: -10kV, 40mA/30msec
Mass Range	*m/z* 35-800
Data processing conditions	
Software	msFineAnalysis (JEOL Ltd.)
Library database	NIST17
Tolerance	±5mDa
Electron	Odd
Element set	C: 0-50, H: 0-100, O: 0-10

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Measured Results

TICC Comparison

Figures 1 and 2 show the TICC data acquired by EI and FI, respectively. In the pyrolysis analysis of the acrylic resin, highly intense peaks for methyl acrylate (MA) and methyl methacrylate (MMA) were observed early in the measurements. Later, multiple dimers and trimers were observed at 10 and 17 minutes, respectively. When nitrogen gas was used, because the column flow rate was reduced, the retention time was increased by approximately 1 minute for most peaks while chromatographic peak separation was comparable to that of helium. The level of sensitivity, one of the concerns for nitrogen as a carrier gas, was also comparable in some cases while reduced to approximately ½ in other cases. This relatively small drop in EI sensitivity is likely related to the open design of the EI/FI ion source (reduced space charging) while FI does not efficiently ionize the nitrogen carrier.

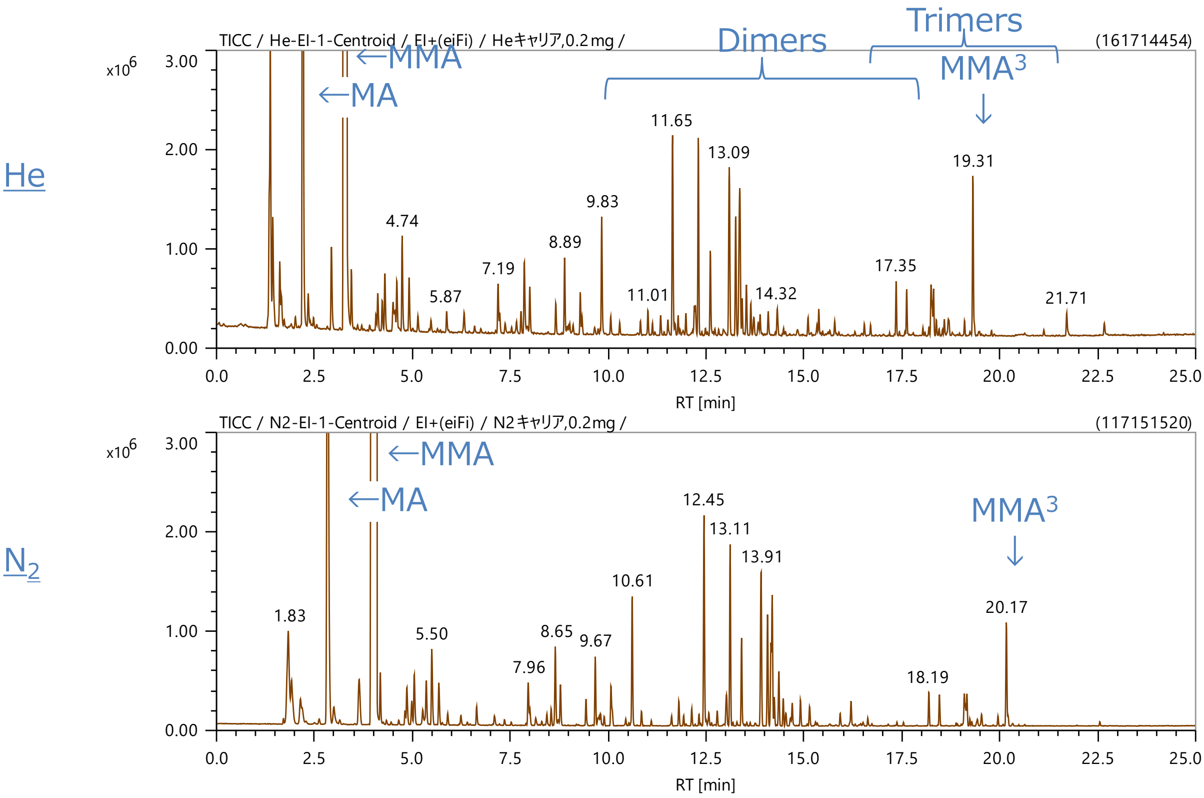

Figure 1. TICC data acquired by EI

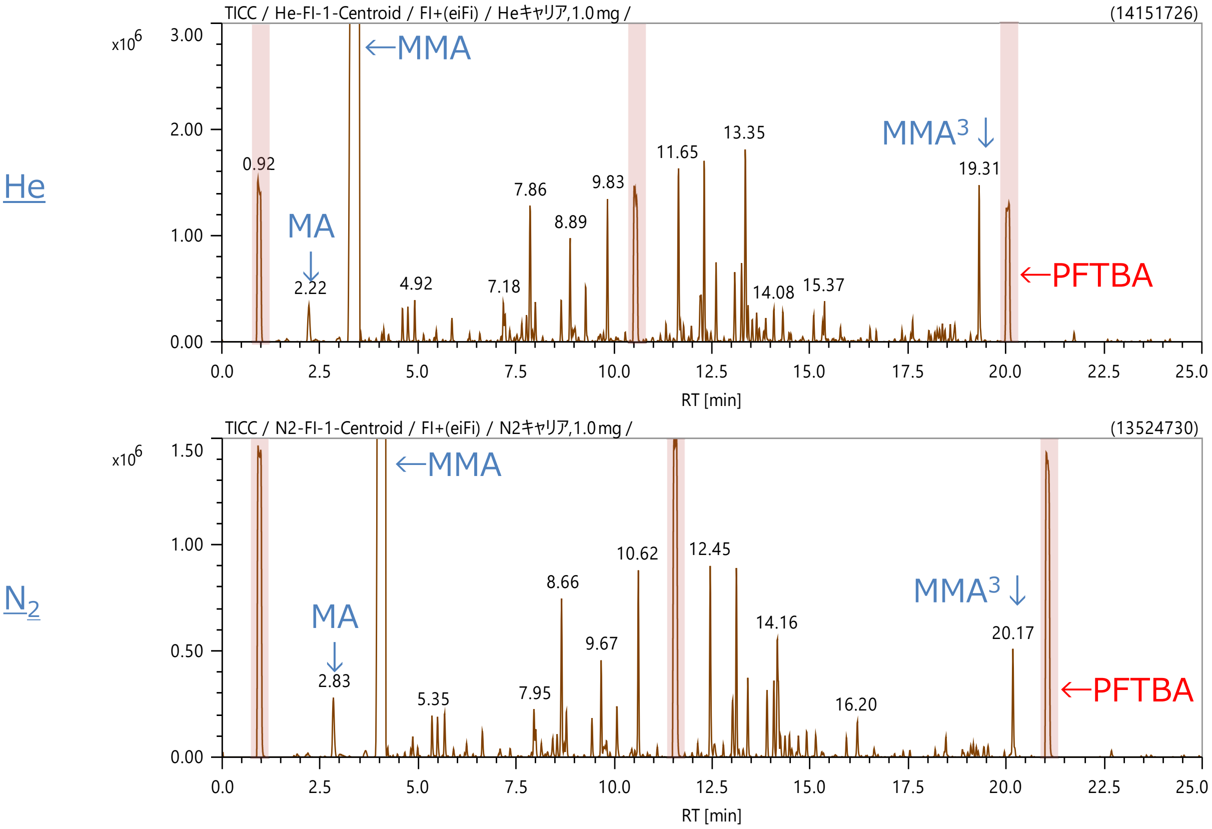

Figure 2. TICC data acquired by FI

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EIC Comparison

Figure 3 shows the EIC of the base ion of MMA^3, a typical trimer, acquired by EI and FI, respectively. In the EIC data, the EI peak shape was nearly equal between helium and nitrogen carrier gases. Peak intensity and S/N declined to less than ½ when nitrogen was used with FI.

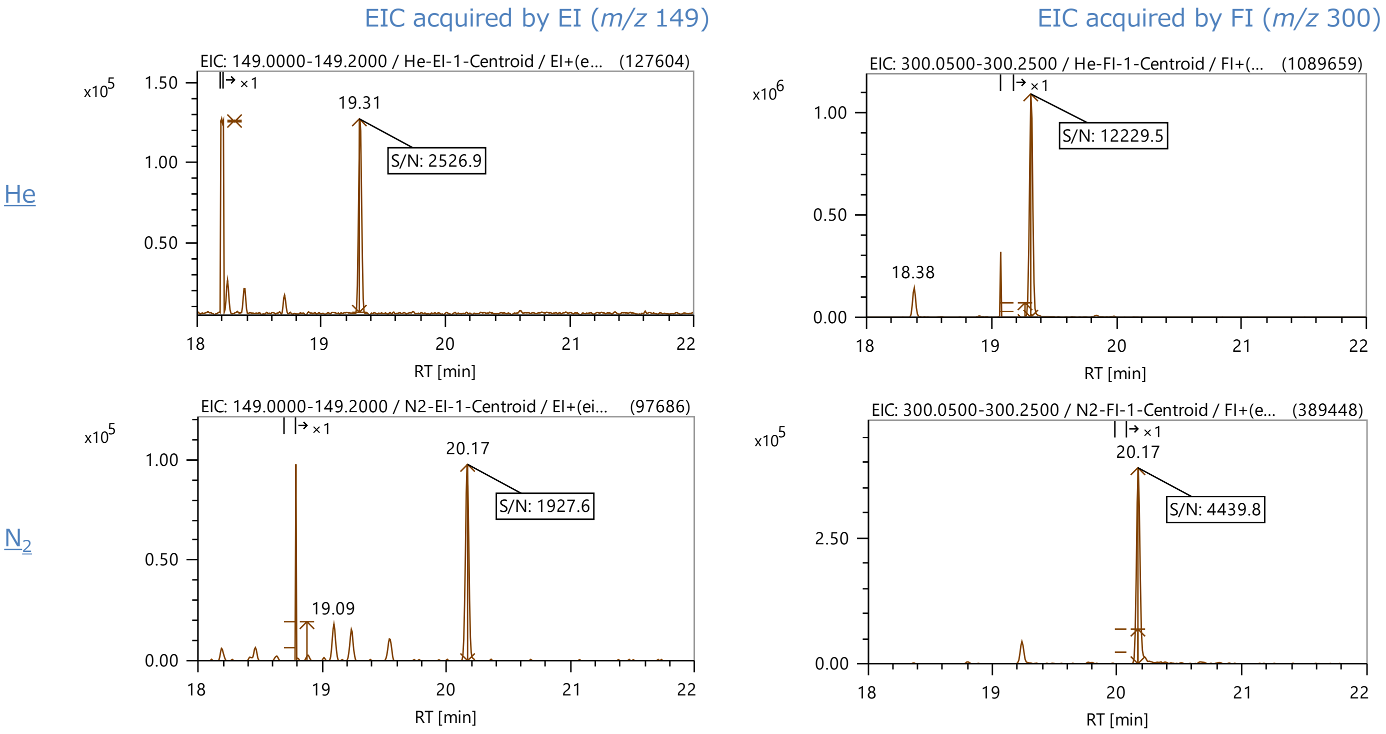
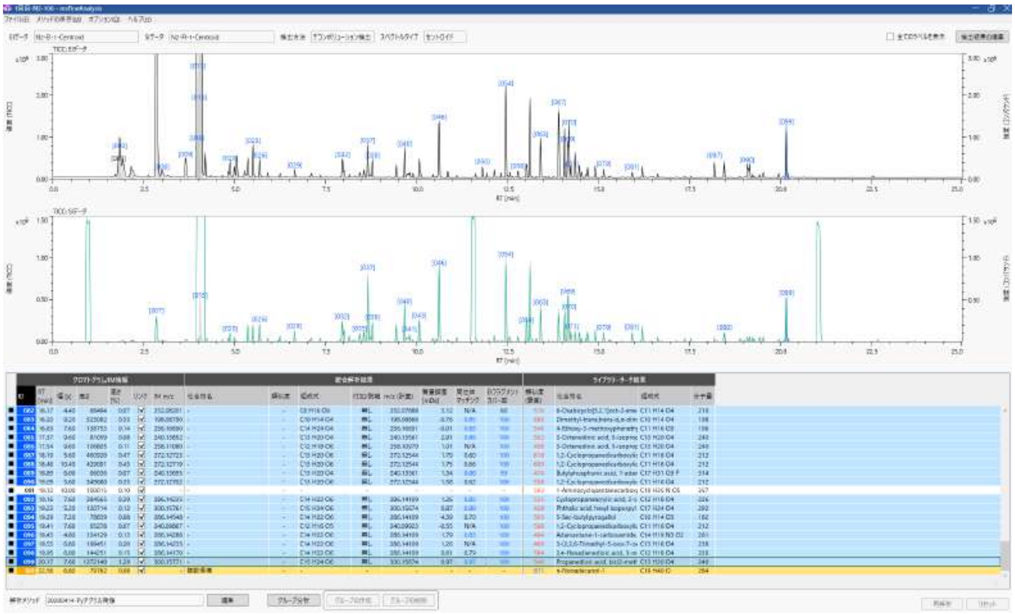


Figure 3. EIC data of MMA^3

Results of msFineAnalysis

Figure 4 is a screen shot of msFineAnalysis integrated analysis results. The top views show the TICCs acquired by EI and FI. The peak list at the bottom shows the estimated molecular formulas that are automatically color-coded according to the number of candidates, thus simplifying the output view for the analyst.



EI
TICC and compound peak

FI
TICC and compound peak

Peak list : The color
depends on analysis results

Figure 4. Screen shot of msFineAnalysis (results of N_2 carrier)

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Compound peaks

msFineAnalysis performs peak assignments based on the results of peak deconvolution. Figure 5 shows the TICC data and compound peaks acquired by EI. Because the retention times are different between helium and nitrogen, overlapping peaks in the TICC data were separated slightly differently. However, peak deconvolution separated these overlapping peaks, making peak assignment possible. The msFineAnalysis auto analysis results showed that 92 peaks out of the top 100 most intense peaks were identified as the same components between helium and nitrogen. The remaining peaks were low in intensity and were identified slightly differently between helium and nitrogen. However, the overall results were comparable.

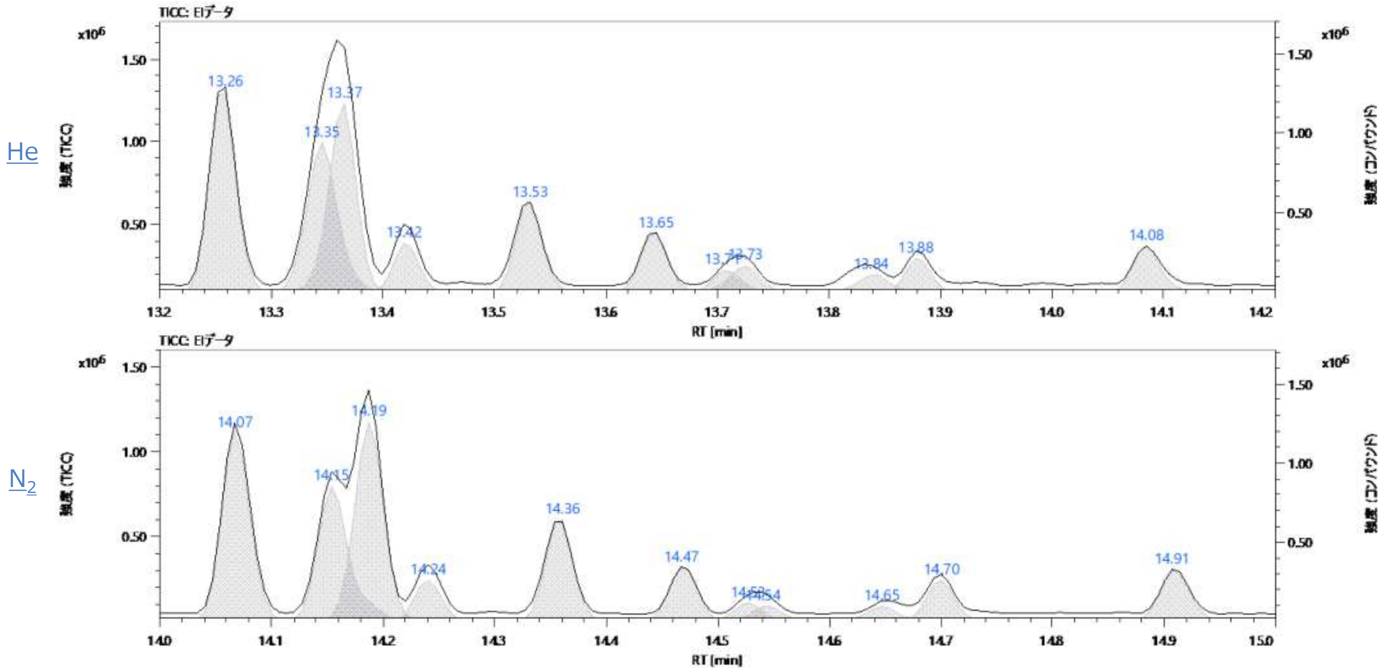

Figure 5. TICC data and compound peaks acquired by EI

Number distribution of estimated molecular formulas

Figure 6 shows the number distribution of estimated molecular formulas for the top 100 peaks. The graph reflects the color-coding used by msFineAnalysis according to the number of candidates for estimated molecular formula assigned to each peak: blue for one candidate; orange for multiple candidates; and gray for none. Results were nearly equal between helium and nitrogen. In both cases, molecular formulas for ~90% of the peaks were uniquely determined.

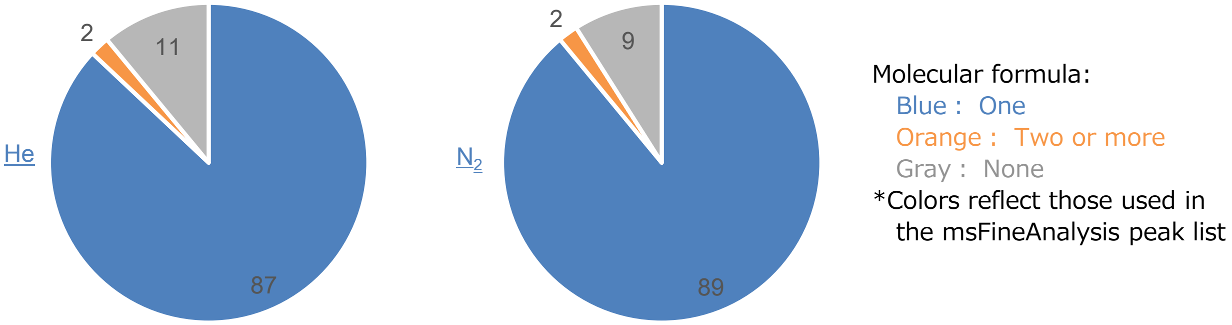

Figure 6. Number distribution for the estimated molecular formulas

Summary

An acrylic resin sample was measured by using pyrolysis/GC-HRTOFMS with helium and nitrogen as the GC carrier gas, and the results were compared to each other. Peak shape and chromatographic separations were equal between helium and nitrogen. The level of sensitivity, one of the concerns when using nitrogen as a GC carrier gas, was equal to or reduced by approximately ½ when the EI/FI ion source was used for the measurements.

msFineAnalysis was used to estimate molecular formulas for the top 100 peaks. The number of candidates identified was nearly equal between helium and nitrogen, suggesting that mass accuracy was not compromised when nitrogen was used as the GC carrier gas. These results demonstrate that exact mass analysis using our GC-TOFMS was effective when using nitrogen as the carrier gas.

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Applications Note

AccuTOF-GCx Series

Comparison of performance between PI and FI by using GC-HRTOFMS

Introduction

Electron ionization (EI) is a common ionization technique for gas chromatography/mass spectrometry (GC/MS). However, EI often does not produce strong molecular ions ($M^{+\cdot}$) because the excess energy generates fragment ions during the ionization process.

The detection of the molecular ion is very important for confirming the molecular weight of the target compounds. Therefore, a soft ionization technique is often necessary to determine the molecular weight information.

Field ionization (FI) is well known as one of the softest ionization techniques commercially available. Similarly, photoionization (PI) can produce molecular ions. In this application note, the characteristics of PI and FI were investigated by using various compounds. Furthermore, the performance between EI, FI and PI for these compounds in diesel fuel were investigated.

Experiment

All samples were analyzed by using JMS-T100GCV (JEOL Ltd.) with the optionally available EI/FI combination ion source and PI ion source. EI data was acquired by using the standard 70eV ionization energy with the EI/FI combination source. FI data was acquired by using a 5μm carbon emitter (Carbotec Analytik) with the combination EI/FI source. A deuterium lamp with a magnesium fluoride window (Hamamatsu Photonics K.K.) was used as the PI source. This lamp has an irradiation wavelength range from 115 to 400 nm (equivalent to the energy range from 3.1 to 10.7 eV) in which the maximum radiation intensity is at 160 nm (7.7 eV). And finally, the EI, FI and PI measurements were done with the same detector voltage so that the peak intensities could be directly compared for each ionization method.

Table 1. Measurement Conditions

Instrument	JMS-T100GCV *"AccuTOF GCv 4G"* (JEOL Ltd.)
Inlet temp.	280˚C
Injection mode	Standard reagents : Split 30:1 Diesel fuel : Split 100:1
Column	ZB-5MSi, 30 m x 0.25 mm, film thickness 0.25 μm
Oven temp. program	50˚C (1 min) => 10˚C /min => 320˚C (10min)
Carrier gas	He (Constant flow : 1.0 mL/min)
Sample volume	Standard reagents : 1 μL Diesel fuel : 0.1 μL
Ionization mode	EI(+) : 70 eV, 300 μA PI(+) : D_2 lamp (Hamamatsu Photonics K.K.) FI(+) : -10 kV, 0 mA ; Baked at 8 mA (20 msec) after recording interval
Transfer line temp.	270˚C
Chamber temp.	EI : 300˚C, FI and PI : 100˚C
m/z range	35-800
Data acquisition interval	0.5 sec

Results

- All compounds produced molecular ions by FI.
- Several compounds produced very low intensity molecular ions by PI.
- Aromatic compounds such as 2,6- dimethyl phenol produced high intensity molecular ions by PI.
- *n*-Octanol produced fragment ions by both PI and FI. These fragment ions were different from the EI fragment ions.
- EI did not show a molecular ion for *n*-octanol.

Various *n*-alkanes and aromatic compounds were detected in the diesel fuel (Fig.2 and Fig.3). The TICC in Figure 2 for EI, FI and PI show the *n*-alkanes as the highest intensity peaks (blue circles) observed in the diesel fuel. Additionally, the average FI mass spectrum in Figure 3 shows high intensity molecular ions for the *n*-alkanes. However, the average PI mass spectrum showed higher relative intensity molecular ions for the aromatic compounds than for the *n*-alkanes. Even so, more fragmentation was observed in the average PI mass spectrum relative to the average FI mass spectrum. The average EI mass spectrum for the diesel fuel was dominated by hydrocarbon fragments in the low mass region.

Conclusion

FI showed strong molecular ions for all compounds measured in this application. PI also showed molecular ions but also produced more fragment ions relative to FI. Also, PI is sensitive for the measurement of aromatic compounds, making it particularly useful for looking at polycyclic aromatics. This application note confirms that PI and FI are soft ionization techniques that can be used to complement the EI results.

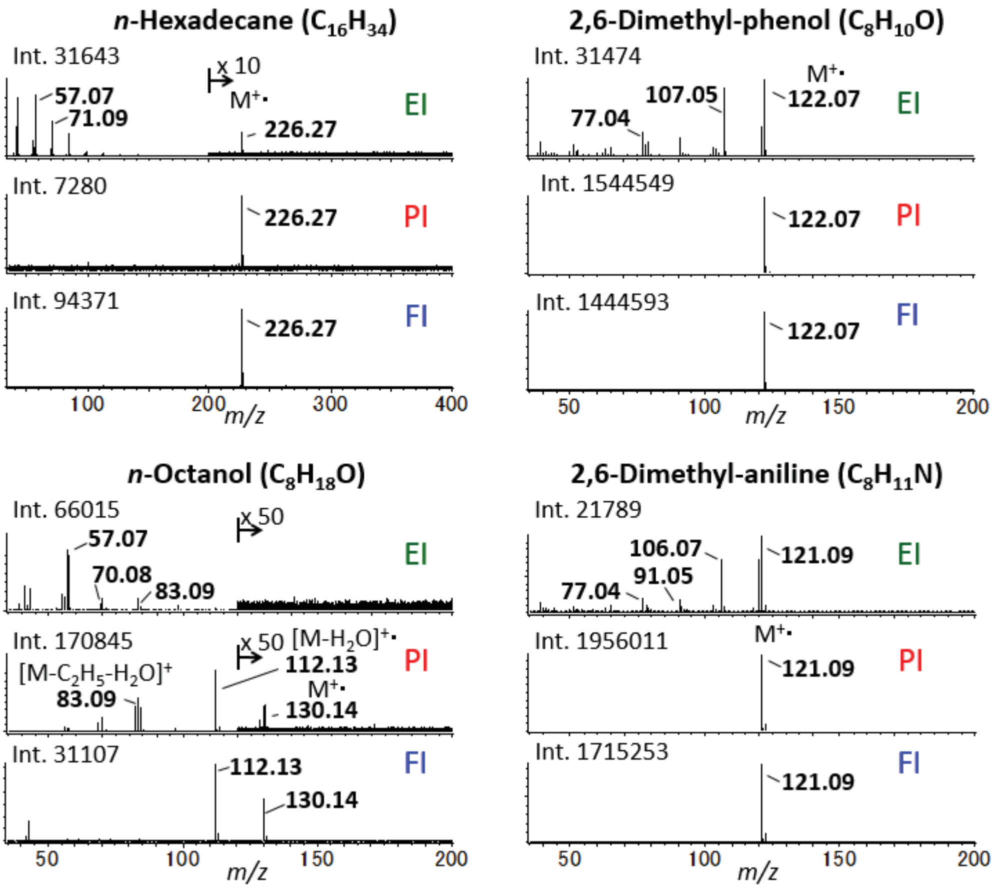


Fig. 1. Compared mass spectra by EI (top), PI (middle) and FI (bottom)

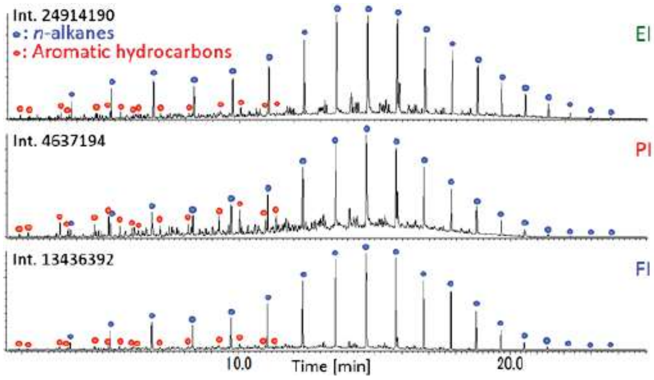
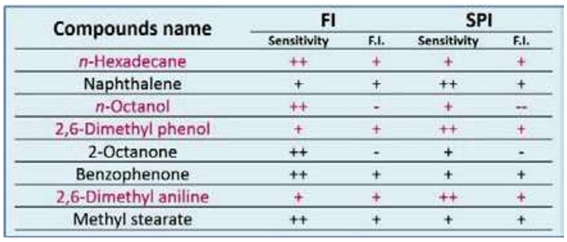


Fig.2. TICC chromatograms of diesel fuel

Table 2. Comparison of the molecular-ion detection by FI and PI

F.I. : Fragment ion



Compounds name	FI		SPI	
	Sensitivity	F.I.	Sensitivity	F.I.
n-Hexadecane	++	+	+	+
Naphthalene	+	+	++	+
n-Octanol	++	-	+	--
2,6-Dimethyl phenol	+	+	++	+
2-Octanone	++	-	+	-
Benzophenone	++	+	+	+
2,6-Dimethyl aniline	+	+	++	+
Methyl stearate	++	+	+	+

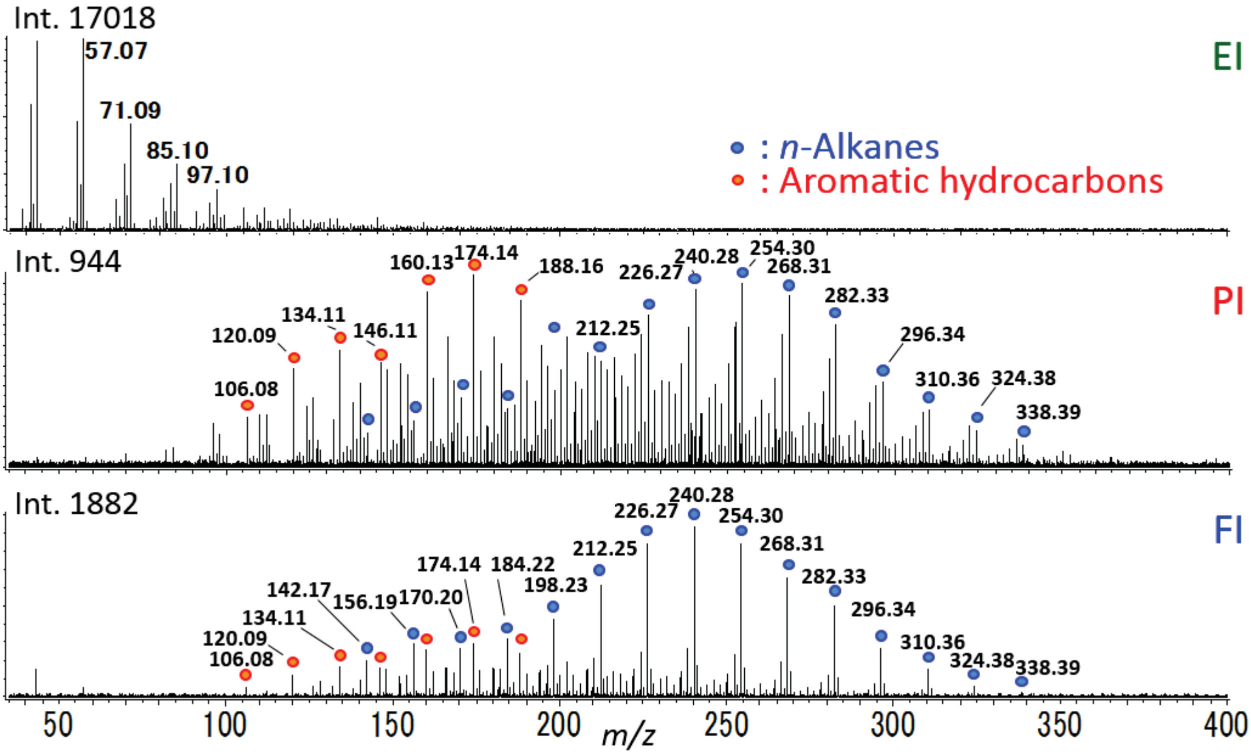
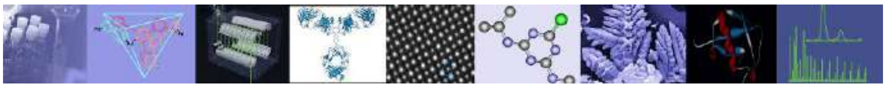


Fig.3. Average mass spectra of diesel fuel



JEOL

Applications Note

AccuTOF-GCv Series

The Qualitative Analysis of an Antioxidant Additive Using the Full Capabilities of the EI/FI/FD Combination Ion Source

Introduction
JEOL has developed a unique EI/FI/FD combination ion source for the “AccuTOF GCv 4G”, a high-resolution GC-time-of-flight (TOF) MS system. This unique ion source provides the capabilities of GC/EI, GC/FI and FD measurements without having to break vacuum in order to switch between each ionization mode. Additionally, this combination is particularly powerful in that it provides library searchable fragmentation information by using EI and high mass accuracy molecular ion information by using FI and FD. In this work, we measured an antioxidant additive by using each ionization mode available on the AccuTOF GCv 4G combination ion source (EI/FI/FD).

Experimental
Sample information and measurement condition are shown in Table 1.

Results
The GC/EI and GC/FI total ion chromatograms (TICs) for the antioxidant sample are shown in Figure 1. Both chromatograms showed the presence of 8 components in the sample. The corresponding EI and FI mass spectra for each component are shown in Figure 2 and Figure 3.

The FI mass spectra for each of the 8 components showed very simple mass spectra that were dominated by their molecular ions. Additionally, the exact masses measured for these compounds showed that there were several isomers present in the antioxidant additive—(A) one at *m/z* 225, (B) three at *m/z* 281, (C) two at *m/z* 337, and (D) two at 393. The accurate mass and calculated elemental composition results are shown in Table 2. The ions generally showed good mass accuracy with less than 1 mDa for both EI and FI mode.

Condition	Measurement		
	GC/EI	GC/FI	FD
Sample	Antioxidant additive		
Concentration	100 ng/uL		10 ug/uL

GC-TOFMS system	AccuTOF GCv 4G (JEOL)		
Ion source	EI/FI/FD combination ion source		
Ionization mode	EI+	FI+	FD+
Ionization condition	70 eV, 300 uA	-10 kV, 45 mA (30 msec refresh between every stored spectrum)	-10 kV, 0 mA → 51.2 mA/min → 45mA
m/z range	*m/z* 35-800		*m/z* 35-1600

GC column	DB-5ms, 30 m x 0.25 mm, 1.0 um	
Inlet mode	Splitless	
Oven temp.	35 C(2min) → 10 C/min → 300 C(22 min)	

Table 1. Measurement condition.

MS061813

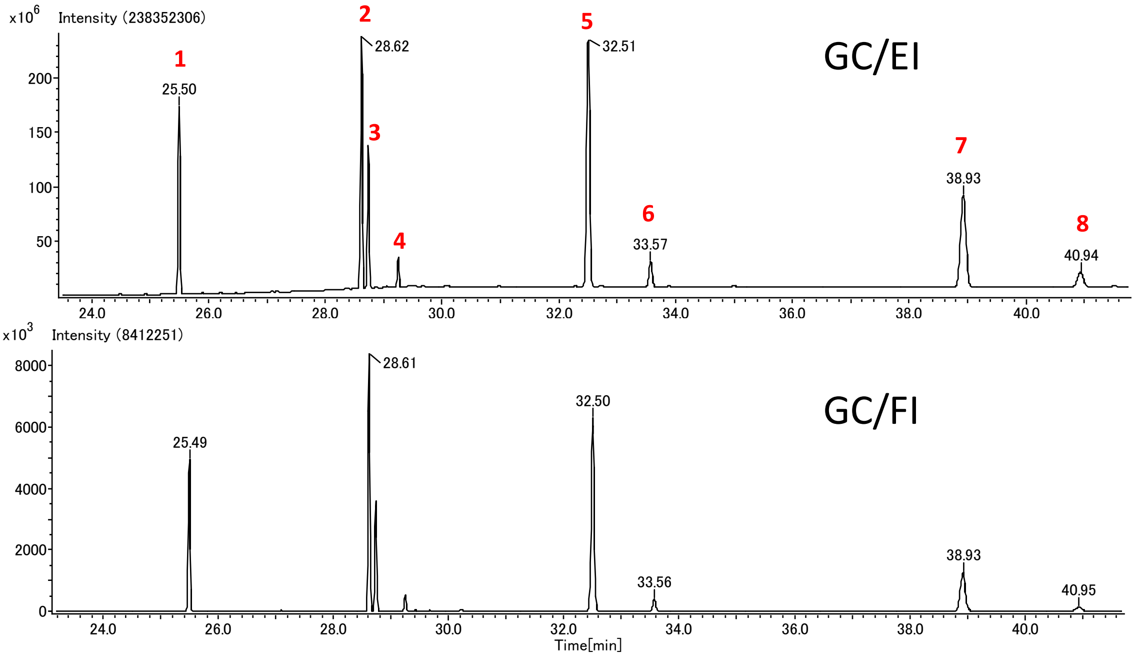


Figure 1. The TICs for GC/EI and GC/FI

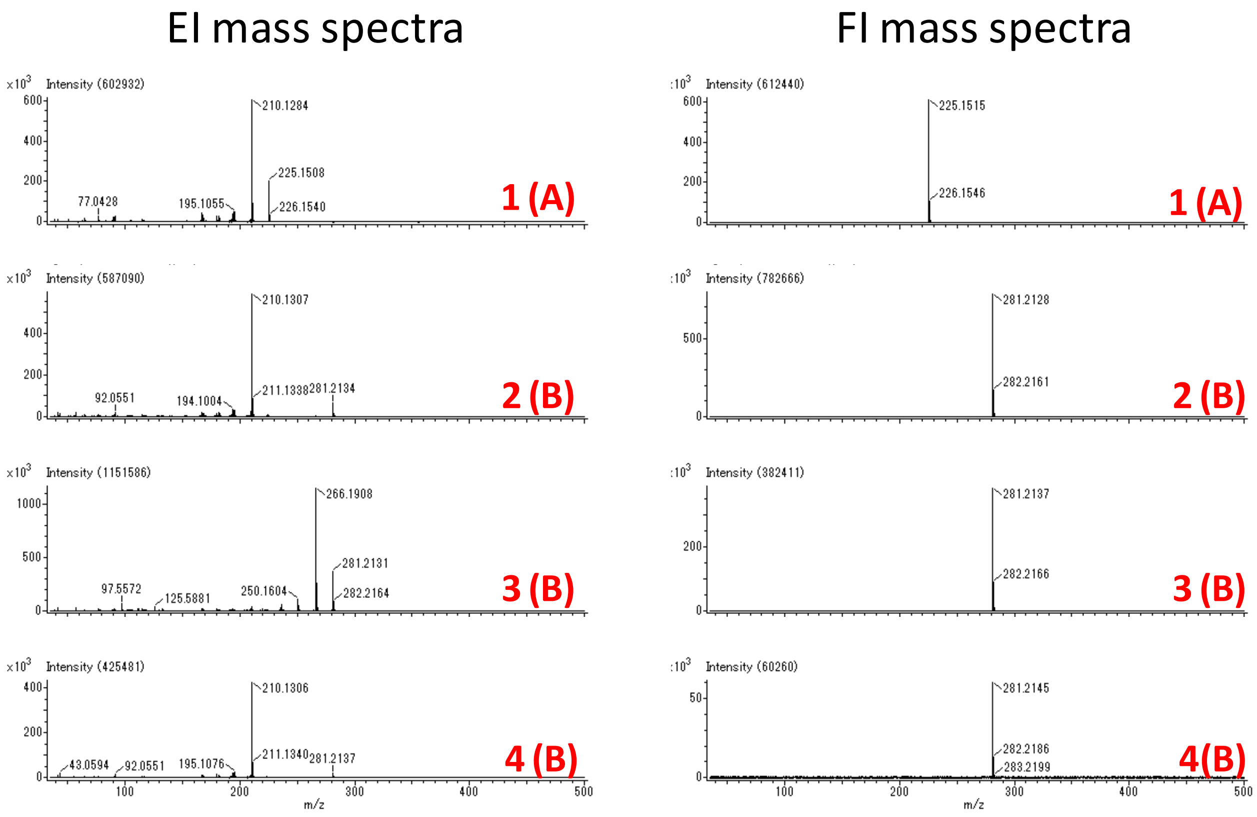


Figure 2. Mass spectra of component1-4, left: EI mass spectra, right: FI mass spectra (A,B: Isomer group)

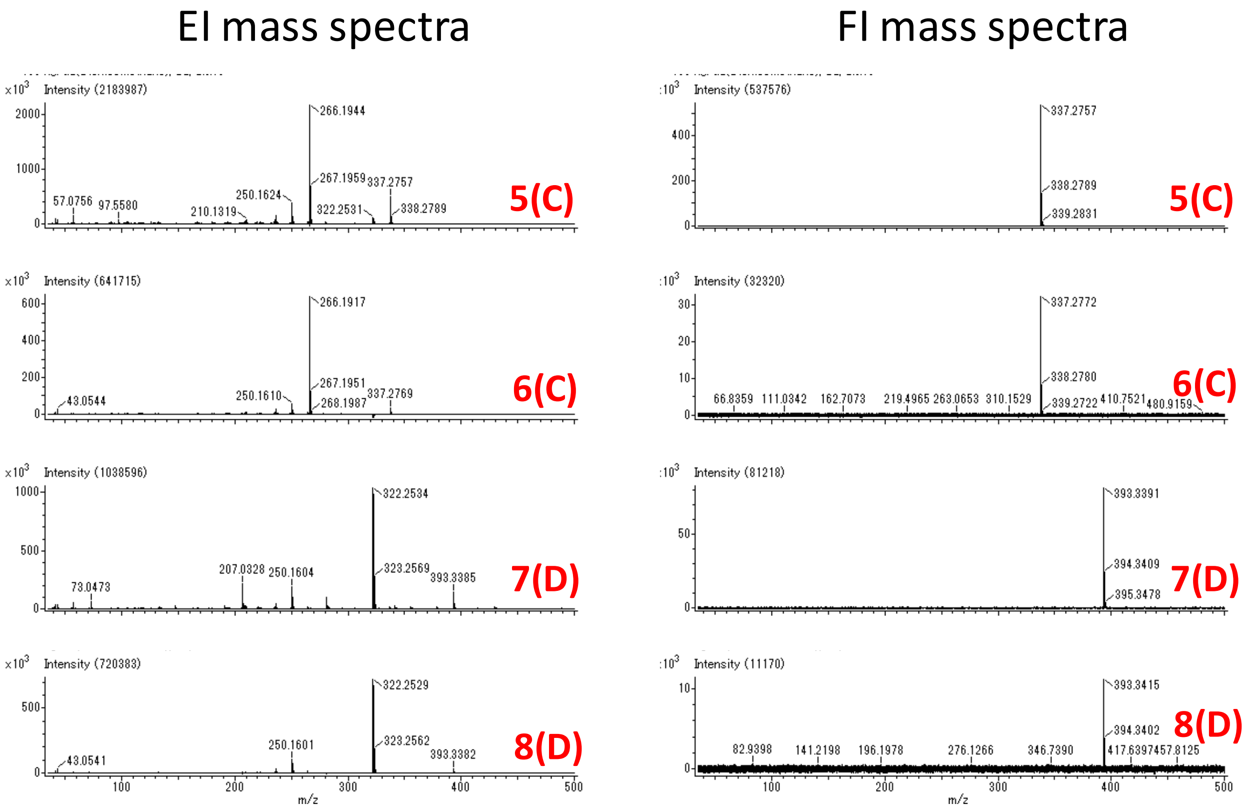
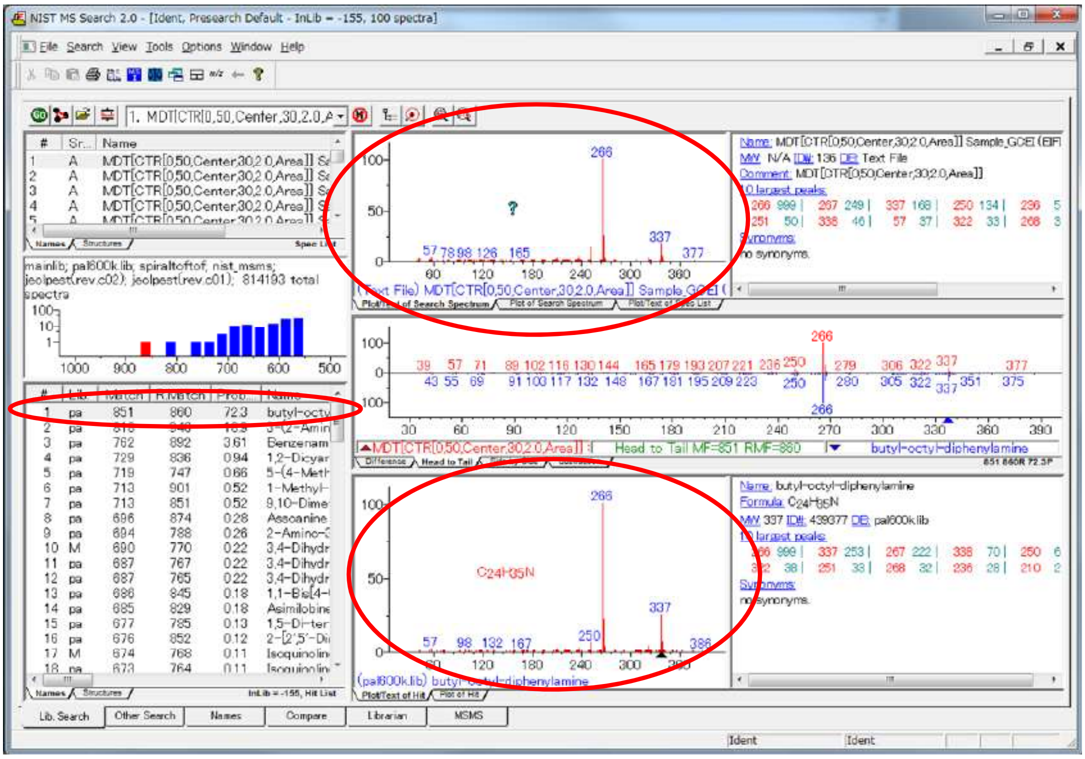


Figure 3. Mass spectra of component5-8, left: EI mass spectra, right: FI mass spectra (C,D: Isomer group)

Component (Isomer group)	Ionization mode	Obs. *m/z*	Theo. *m/z*	Error (mDa)	Fomula
1(A)	EI	210.1284	210.1283	0.1	$C_{15}H_{16}N$
		225.1508	225.1518	−1.0	$C_{16}H_{19}N$
	FI	225.1515	225.1518	−0.3	$C_{16}H_{19}N$
2(B)	EI	266.1908	266.1909	−0.1	$C_{19}H_{24}N$
		281.2131	281.2144	−1.3	$C_{20}H_{27}N$
	FI	281.2137	281.2144	−0.6	$C_{20}H_{27}N$
5(C)	EI	266.1917	266.1909	0.8	$C_{19}H_{24}N$
		337.2769	337.2770	−0.1	$C_{24}H_{35}N$
	FI	337.2772	337.2770	0.3	$C_{24}H_{35}N$
7(D)	EI	322.2534	322.2535	−0.1	$C_{23}H_{32}N$
		393.3385	393.3396	−1.1	$C_{28}H_{43}N$
	FI	393.3396	393.3396	−0.4	$C_{28}H_{43}N$

Table 2. Accurate mass measurement results


NIST MS Search 2.0 - [Ident, Presearch Default - InLib = -155, 100 spectra]
File Search View Tools Options Window Help
1. MDT[CTR[0.50,Center,30,2.0,A
Names Structures
Spec List
Plot/Text of Search Spectrum
Plot of Search Spectrum
Plot/Text of Spec List
Difference
Head to Tail
Plot/Text of Hit
Plot of Hit
Lib. Search
Other Search
Names
Compare
Librarian
MSMS
Ident
C24H35N
266
337
377
250
386
Head to Tail MF=851 RMF=860
butyl-octyl-diphenylamine
851 860R 72.3P

mainlib; pal600k.lib; spiraltoftof; nist_msms; jeolpest(rev.c02); jeolpest(rev.c01); 814193 total spectra

#	Lib	Match	R.Match	Prob.	Name
1	pa	851	860	72.3	butyl-octy
2	pa	[illegible]	[illegible]	[illegible]	3-(2-Amin
3	pa	762	892	3.61	Benzenam
4	pa	729	836	0.94	1,2-Dicyar
5	pa	719	747	0.66	5-(4-Meth
6	pa	713	901	0.52	1-Methyl-
7	pa	713	851	0.52	9,10-Dime
8	pa	696	874	0.28	Assoanine
9	pa	694	788	0.26	2-Amino-3
10	M	690	770	0.22	3,4-Dihydr
11	pa	687	767	0.22	3,4-Dihydr
12	pa	687	765	0.22	3,4-Dihydr
13	pa	686	845	0.18	1,1-Bis[4-
14	pa	685	829	0.18	Asimilobine
15	pa	677	785	0.13	1,5-Di-ter
16	pa	676	852	0.12	2-[2',5'-Di
17	M	674	768	0.11	Isoquinolin
18	pa	673	764	0.11	Isoquinolin

InLib = -155, Hit List

Name: MDT[CTR[0,50,Center,30,2.0,Area]] Sample_GCEI (EIF

MW: N/A ID#: 136 DB: Text File

Comment: MDT[CTR[0,50,Center,30,2.0,Area]]

10 largest peaks:

266 999 | 267 249 | 337 168 | 250 134 | 236 5

251 50 | 338 46 | 57 37 | 322 33 | 268 3

Synonyms:

no synonyms.

Name: butyl-octyl-diphenylamine

Formula: $C_{24}H_{35}N$

MW: 337 ID#: 439377 DB: pal600k.lib

10 largest peaks:

266 999 | 337 253 | 267 222 | 338 70 | 250 6

322 38 | 251 33 | 268 32 | 236 28 | 210 2

Synonyms:

no synonyms.

Figure 4. NIST search for component 5.

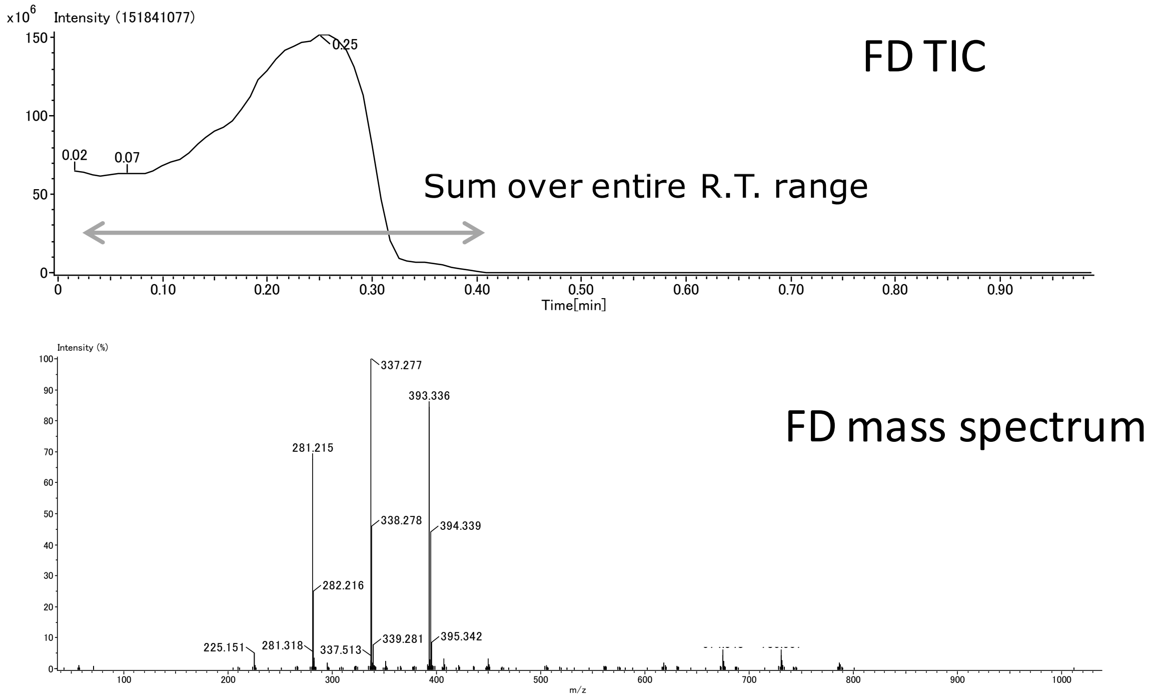


Figure 5. FD measurement result

As an example of how the EI data is library searchable, the EI spectrum for component 5 was exported to the NIST database which in turn showed that the top candidate for this EI fragmentation pattern is butyl-octyl-diphenylamine (Figure 4). To further support this match, the elemental composition of this compound ($C_{24}H_{35}N$) exactly matches the composition identified through the EI and FI accurate mass measurements. Moreover, butyl-octyl-diphenylamine is an antioxidant which further supports this identification for component 5.

Next, the same antioxidant additive mixture was measured using FD mode, in which the sample is loaded directly onto the emitter probe. Figure 5 shows both the TIC and mass spectrum for this analysis. The measurement was completed within 1 minute and confirmed that the same four compositions were observed in this experiment (*m/z* 225.2, 281.2, 337.3 and 393.3) as were observed in the GC/EI and GC/FI analyses. Additionally, the dimers for several of these ions were also observed in the mass spectrum. While FD is not able to determine the presence of multiple isomers (like the chromatography techniques), the analysis speed (less than 1min) is very useful for quickly evaluating the types of constituents that are present in a given sample.

Conclusion

In this work, we showed a brief study for an antioxidant additive using each ionization mode available on the AccuTOF GCv 4G EI/FI/FD combination ion source. Furthermore, each technique was accessed without changing out the ion source or breaking vacuum. The EI/FI/FD combination ion source used in conjunction with the high resolution capabilities of the AccuTOF GCv 4G is a powerful tool for doing chemical qualitative analysis.

Applications note

MS
MSTips No. 453
GC-TOFMS Application

Structure Analysis of Unknown Compound in Lemon Juice by SPME-GC-TOFMS and msFineAnalysis AI

Product used: Mass Spectrometer (MS)

Introduction

Food flavor components are known to be an important element of good taste. Gas chromatography-mass spectrometry (GC-MS) is often used to analyze food flavor components. This is because food flavors are highly volatile and complex with numerous components. Electron ionization (EI) is one of the most popular ionization methods used in GC-MS. Consequently, compounds are typically identified by a mass spectral database search using EI mass spectra. Because molecular ions are often weak or absent in 70 eV EI mass spectra, identification of unknowns can be difficult by EI alone. In these cases, soft ionization (SI) can be very helpful for producing and identifying molecular ions. We had developed an integrated qualitative analysis workflow that automatically combines and interprets the information from EI and SI data1). And then in 2018, we introduced our integrated qualitative analysis software “msFineAnalysis” which uses both EI and SI data to improve compound identification for GC-MS applications. Despite the fact that msFineAnalysis was automatically able to determine the molecular formula and partial structure information from EI fragment ion formulas, the actual structural formulas still required manual analysis using chemical compositions. To address this, we then developed an automated structure analysis software package named “msFineAnalysis AI” which uses artificial intelligence (AI) to predict EI mass spectra from chemical structures2). We have used our newly-developed AI model to create a database of predicted EI mass spectra for around 100 million compounds.

In this MSTips, we report on an example of structure estimation of an unknown compound in lemon juice using msFineAnalysis AI.

Experimental

American lemon juice obtained by squeezing was used as sample. Lemon juice 10 mL was sealed in a 20 mL vial. The SPME mode of the HT2850T autosampler (HTA S.R.L.) was used as the sample preparation device, and volatile components in the headspace area of the vials were targeted for the measurement. A GC-TOFMS (JMS-T2000GC AccuTOF™ GC-Alpha, JEOL Ltd.) was used for the measurement. We performed HS-SPME-GC-TOFMS measurements using both EI and field ionization (FI) modes with a combination EI/FI/FD ion source. The qualitative data processing was performed with msFineAnalysis AI (JEOL Ltd.). Measurement conditions are shown in Table 1.

JMS-T2000GC AccuTOF™ GC-Alpha

HT2850T

Table 1 Measurement

SPME	
SPME Fiber	DVB/CAR/PDMS 2mm (Merck)
Sample amount	10 mL
Extraction temp.	60 °C
Extraction time	30 min
Desorption time	3 min

GC	
Column	ZB-WAX（Phenomenex） 30 m×0.25 mm I.D., df=0.25 μm
Inlet	250 °C, EI=Split 20:1, FI=Splitless
Oven	40 °C（2 min）→10 °C/min→250 °C/min (1 min)
Carrier flow	He, 1.0 mL/min（Constant Flow）

MS	
Ion Source	EI/FI/FD combination ion source
Ionization	EI+:70 eV, 300 μA, FI+:-10 kV, 40 mA
m/z Range	*m/z* 35 - 800

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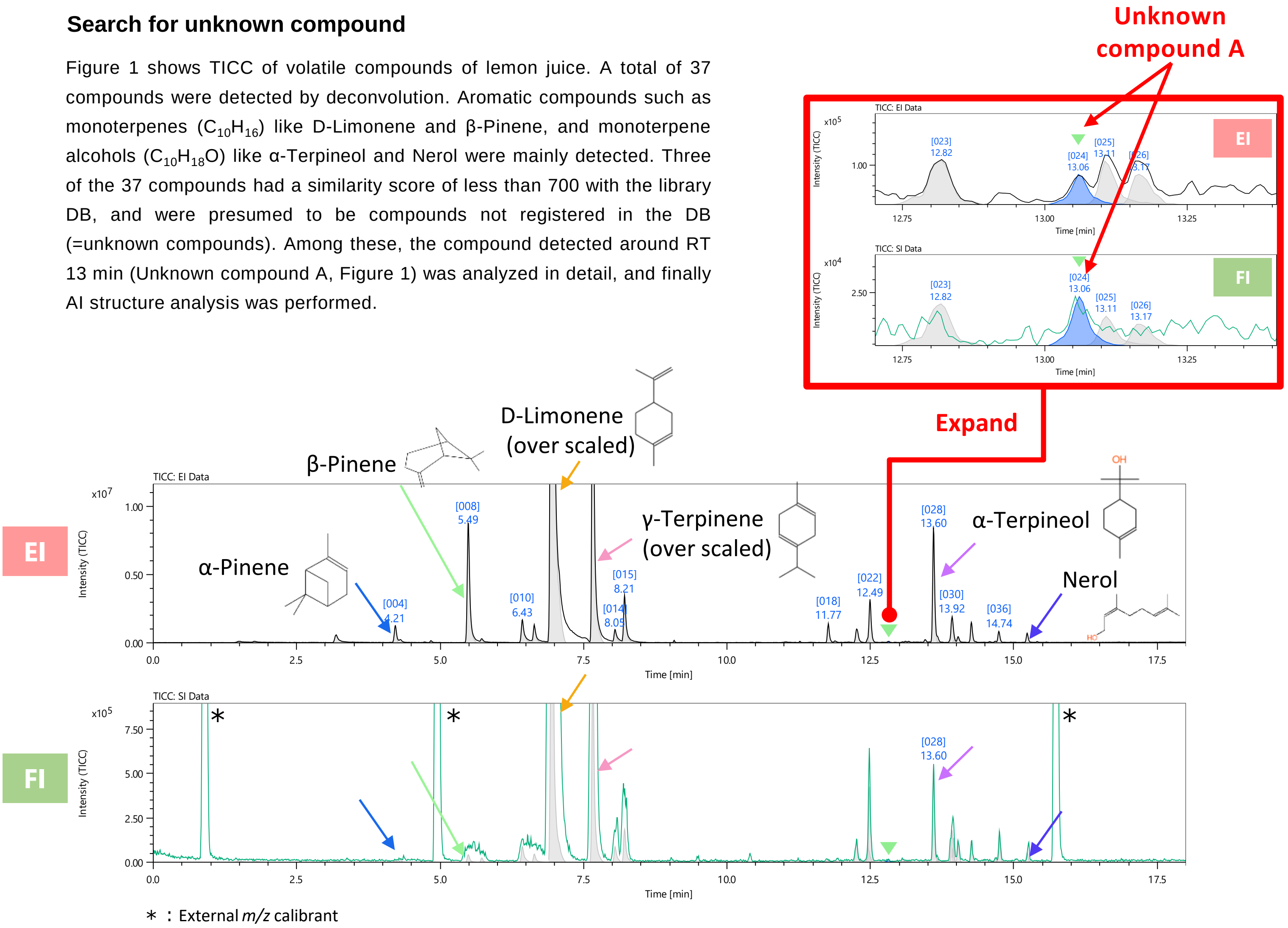
Results and Discussion

Search for unknown compound

Figure 1 shows TICC of volatile compounds of lemon juice. A total of 37 compounds were detected by deconvolution. Aromatic compounds such as monoterpenes ($C_{10}H_{16}$) like D-Limonene and β-Pinene, and monoterpene alcohols ($C_{10}H_{18}O$) like α-Terpineol and Nerol were mainly detected. Three of the 37 compounds had a similarity score of less than 700 with the library DB, and were presumed to be compounds not registered in the DB (=unknown compounds). Among these, the compound detected around RT 13 min (Unknown compound A, Figure 1) was analyzed in detail, and finally AI structure analysis was performed.



＊：External *m/z* calibrant

Figure 1 TICC of volatile compounds of lemon juice

Mass spectra and elemental composition estimation results of unknown compound A

Figure 2 shows mass spectra of unknown compound A. For this compound, molecular ion *m/z* 150 was detected in the mass spectra of both the EI and FI data (IM indicate molecular ion in Figure 2). However, in the FI data, the relative intensity of the molecular ion was high and detected as the base peak. The molecular formula estimated from the molecular ion in the FI mass spectrum was $C_{10}H_{14}O$.

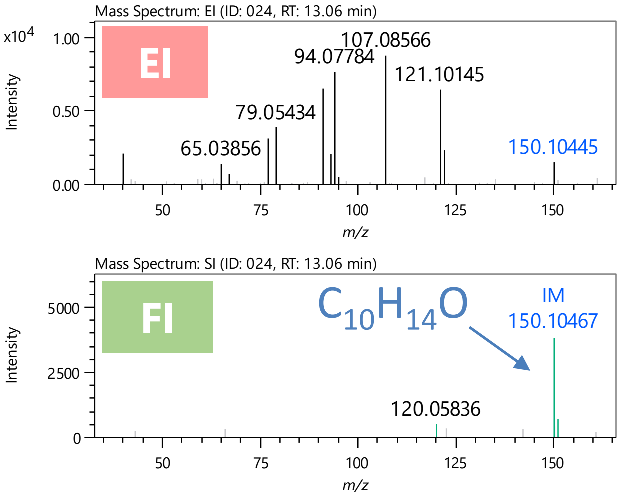


Figure 2 Mass spectra of unknown compound A

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AI structure analysis result

Figure 3 shows AI structure analysis results (Top 18 candidates). Among the wide variety of structural formulas, the No. 1 candidate (with the highest similarity score with the AI library) in the AI structure analysis results was a monocyclic monoterpene aldehyde-like compound (Figure 4). Reference 3 states that "*m/z* 79, 93, 107, and 121 occur as fragment ions characteristic of terpenes and terpenoids," which was confirmed by the EI mass spectrum of this compound (Figure 5). Furthermore, the unknown compound A had a structure similar to safranal, a monocyclic monoterpene aldehyde that is the main component of saffron's aroma (Figure 6). Safranal is known to be produced by hydrolysis of the monoterpene glycoside picrocrocin[4] (Figure 6). Therefore, unknown compound A may be an aroma component as well as safranal.

As shown in the above results, the structure of unknown compound A could be estimated by AI structural analysis.

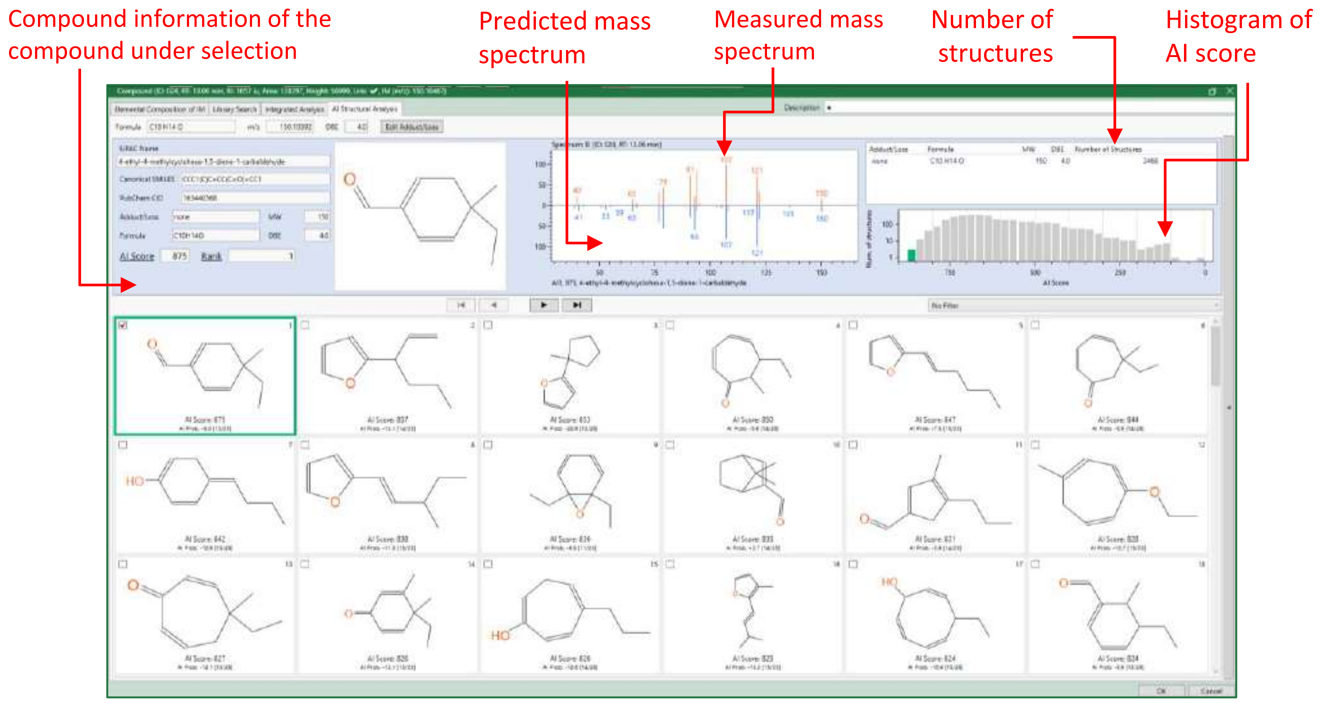

Figure 3 AI structure analysis result of unknown compound A (top 18 candidates)

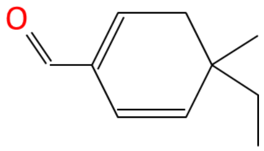

Figure 4 Structural formula of No. 1 candidate of AI structure analysis

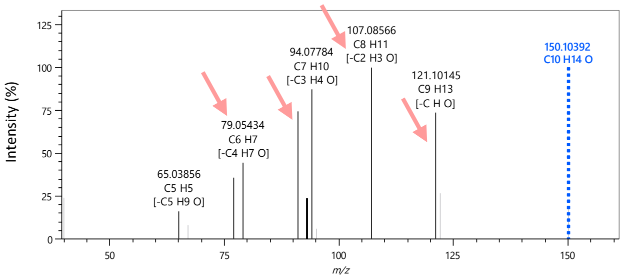

Figure 5 EI mass spectrum of unknown compound A (The blue peak indicates the position of molecular ion)

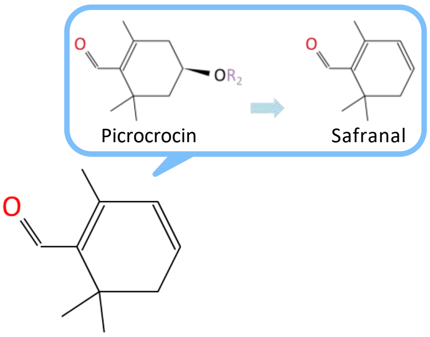

Figure 6 Structural formula of Safranal

Conclusions

In this MSTips, we introduced an example of structure estimation of an unknown compound in lemon juice using msFineAnalysis AI. Manual structure analysis requires a lot of mass spectrometry knowledge and analysis time, but msFineAnalysis AI enables rapid structural estimation. This software is expected to be used for the analysis of unknown compounds in food flavor using GC-TOFMS.

Reference

1) M. Ubukata et al, Rapid Commun Mass Spectrom. **2020**; 34:e8820.
2) A. kubo et al, *Mass Spectrometry*, **2023**, *12*, A0120.
3) Written by F.W. McLafferty. Translated by T. Ueno., Interpretation of Mass Spectra. (Written in Japanese)
4) Kobe Pharmaceutical University, Medicinal Botanical Garden Letter. Issued on 11/22/2022. (Written in Japanese)

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Applications note
MS
MSTips No.449
GC-TQMS Application

Comparison of aromatic compounds in two different red wines by using GC–High resolution TOFMS with the integrated data analysis of msFineAnalysis AI

Related products: Mass Spectrometer (MS)

Introduction

It is known that the taste of alcoholic beverages such as wine has a strong influence from aromatic compounds. It is also known there are many common aromatic compounds that contribute to the typical taste and aromas of wine independent of the production regions, and there are also many aromatic compounds that are strongly dependent on the production regions and the kinds of grapes.

In case of wine, the difference of taste and aroma between the wines that were brewed the different production region is often discussed. It is of interest to know the type of aromatic compounds that contribute to those differences.

The combination of the micro solid-phase extraction (SPME) and gas chromatograph-mass spectrometer (GC-MS) is widely used for the analysis not only of aromatic compounds but also volatile organic compounds. The combined analysis method was used to detect the characteristic compounds of two type of red wines, Bordeaux and Bourgogne. All aromatic samples were measured by not only electron ionization (EI) but also photoionization (PI) to identify the characteristic chemical compounds that were included in the only Bordeaux or only Bourgogne wine. In addition, both measurement data were analyzed by using msFineAnalysis AI software. The characteristics chemical compounds that differ between the two wines were identified by using the differential analysis functionality of msFineAnalysis AI.

JMS-T2000GC AccuTOF™ GC-Alpha

Experiment

Two typical red wines, produced in Bordeaux and Bourgogne, which are generally available in a supermarkets were used as test samples. 2 mL portion of the wine samples were added to 15mL glass vial and immediately sealed. The SPME fiber was inserted into the headspace of the glass vial and the fiber was exposed for 20 min at room temperature. All extracted compounds in the SPME fiber were introduced into GC by heating at injection port of GC for 1min. The details of measurement condition were shown in Table 1. To use the differential analysis functionality, each sample was measured for three times using EI ionization. Then those measurement results were applied to msFineAnalysis AI (JEOL Ltd.) together with the measurement results by PI ionization.

Table 1. Measurement condition

GC Condtion	
GC	8890GC (Agilent)
Injection mode	Pulsed Splitless
	Purge Pressure : 100kPa
	Pulsed time : 1.0 min
	Purge Flow : 20mL/min
	Purge On time : 0.9 min
	Septum Purge Flow : 3.0 mL/min
Injection Temp	250°C
Column	InertCap WAX (GL Science)
	30m X 0.25mm(I.D.), 0.25μm Film Thickness
Oven	40°C (3 min) > 7°C/min > 90°C (0min)
	> 20°C/min > 240°C (7.36min)
He Flow	1mL/min (Constant Flow mode)

MS Condition	
Mass Spectrometer	JMS-T2000GC AccuTOF™ GC-Alpha
Ion source	EI/PI Combination Ion Source
Ionizetion	EI+ : 70eV, 300μA
	PI+ : D2 lamp, 115-400nm
Source tem.	250°C
GC Interface temp.	250°C
m/z range	m/z 40 - 550

SPME condition	
Fiber	50/30μm DVB/CAR/PDMS (Gray)
Sampling Method	Headspace
Sample Amaount	2mL
Extraction Temp	Room temperature
Extraction Time	20 min
Desorption time	1 min

Results

Total Ion Current Chromatograms (TICCs) obtained as a result of measuring the aromatic components of Bordeaux and Bourgogne wines by EI ionization are shown in Fig.1. In addition, the volcano plot obtained as a result of performing a difference analysis using Bordeaux wine as Sample A (blue) and Bourgogne wine as Sample B (red) is shown in Fig.2. In the volcano plot of msFineAnalysis AI software, the horizontal axis represents the intensity ratio between two samples (Log2(B/A)), and the vertical axis represents statistical reproducibility (-log10(p-value)). By using this volcano plot, it becomes easy to understand the difference in components. The plots in the area with a blue background shown in Fig. 2 are characteristically detected peaks in Sample A, and conversely, those with a red background are the characteristically peaks in Sample B.

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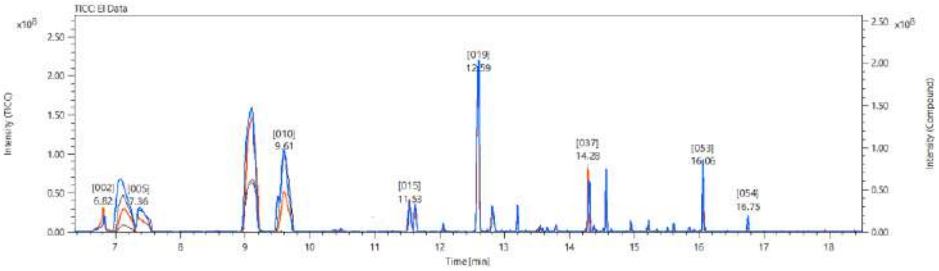

Fig.1 TICC with EI for Sample A(Blue) and B(Red)

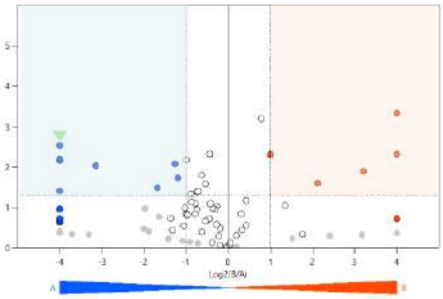

Fig.2 Volcano plot of Bordeaux(Blue) vs. Bourgogne(Red)

Table 2. Integrated qualitative analysis results for the characteristic compounds for Sample A (Bordeaux) and Sample B (Bourgogne)

			ariance Component Analysis Resul			Total Result						
RT [min]	Area	IM m/z	Class	Log2(B/A)	p-value	Compound Name	Similarity	Formula	Calculated m/z	Mass Error [mDa]	Isotope Matching	EI Fragment Coverage
Characteristic Compounds for Sample A												
5.36	59432816	116.08281	A Only	< -4	0.007	Butanoic acid, ethyl ester	731	C6 H12 O2	116.08318	-0.37	0.89	100
10.37	14756626	-	A Only	< -4	0.006	Acetic acid, hexyl ester	909	C8 H16 O2	-	-	-	100
11.07	2354736	-	A Only	< -4	0.003	1-Pentene, 4-methyl-	810	C6 H12	84.09335	-0.72	N/A	100
11.26	3623007	84.09313	A > B	-1.68	0.033	Cyclopropane, propyl-	746	C6 H12	84.09335	-0.22	N/A	100
13.96	2992587	-	A Only	< -4	0.109	Propylene Glycol	840	C3 H8 O2	76.05188	-0.53	0.61	100
15.35	5287901	152.04676	A Only	< -4	0.039	Methyl salicylate	763	C8 H8 O3	152.04680	-0.04	0.91	88
15.61	16329082	-	A > B	-1.26	0.008	Hexanoic acid	865	C6 H12 O2	117.09101	-0.82	N/A	100
15.85	9603910	108.05672	A > B	-3.15	0.009	Benzyl alcohol	942	C7 H8 O	108.05697	-0.24	0.89	100
16.74	27620411	144.11423	A > B	-1.19	0.019	Octanoic acid	949	C8 H16 O2	144.11448	-0.25	0.63	100
Characteristic Compounds for Sample B												
13.27	2658553	-	A < B	3.21	0.013	(S)-3-Ethyl-4-methylpentanol	931	C8 H18 O	-	-	-	100
13.50	5535502	192.15035	B Only	> 4	0.000	2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl-	751	C13 H20 O	192.15087	-0.52	0.81	90
14.28	110243250	200.17694	A < B	1.00	0.005	Decanoic acid, ethyl ester	921	C12 H24 O2	200.17708	-0.15	0.70	95
15.57	2263498	-	B Only	> 4	0.005	Dodecanoic acid, ethyl ester	865	C14 H28 O2	228.20838	0.17	0.81	100
17.93	4687977	206.16637	A < B	2.13	0.025	2,4-Di-tert-butylphenol	945	C14 H22 O	206.16652	-0.14	0.89	100

The characteristic compounds in each sample (Bordeaux : Sample A and Bourgogne : Sample B) were identified and listed in Table 2. Totally 14 compounds (9 from Bordeaux, 5 from Bourgogne) were identified. Concerning 8 of 14 compounds, the molecular ions were identified, and the exact m/z values of those molecular ions agreed with the exact m/z values of the estimated compounds. In addition, the EI fragment coverage values for those compounds that are calculated from the relationship between the elemental composition of fragment ions and those of molecular ion were also high values. This means the validity of the compound identification is much higher than by simple library search alone.

It is known that several ‘carboxylic acid esters’ are the typical aromatic compounds that characterized the aroma of wine. Many kinds of methyl and/or ethyl esters of low fatty acids were detected from both of Bordeaux and Bourgogne wine. This means that the esters of low fatty acids strongly contribute the aroma of wines.

The comparison of TICC by EI and PI for Hexanoic acid ethyl ester that was detected from both of Bordeaux and Bourgogne wines is shown in Fig 3. The position of the Hexanoic acid ethyl ester in the volcano plot is also shown in Fig.4. By using the differential analysis functionality of msFineAnalysis, the detailed data analysis and confirming information is available for each peak.

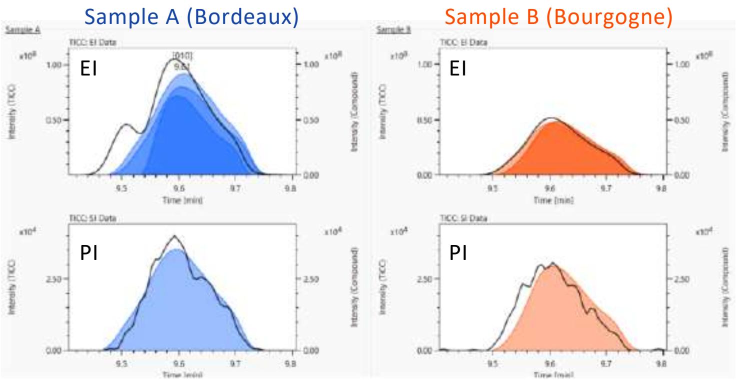

Fig.3 TICC of EI and PI for Hexanoic acid ethyl ester detected in Sample A and Sample B

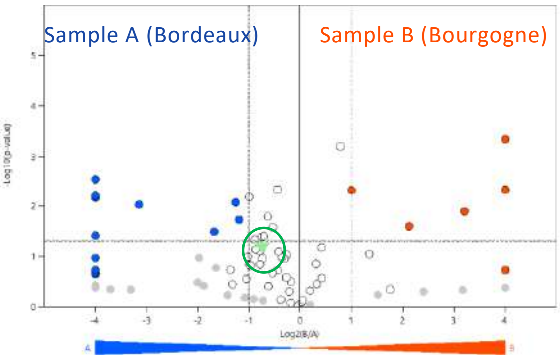

Fig.4 Relative position of Hexanoic acid ethyl ester in Volcano plot

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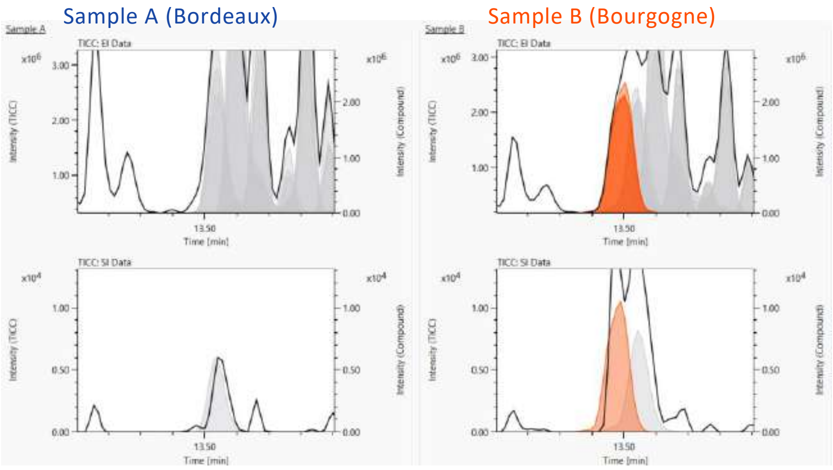

Fig.5 TICC of EI and PI for 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl-2(1H)-Naphthalenone detected only in Sample B (not in Sample A)

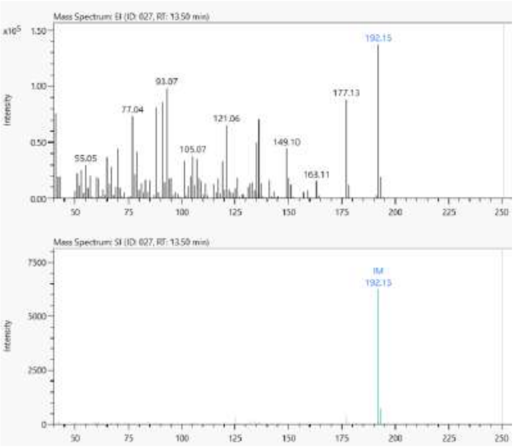

Fig.6 Mass spectrum of 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl-2(1H)-Naphthalenone detected in Sample B

It is interesting to note that esters of fatty acids with a relatively large number of carbon atoms were detected as the characteristic compounds of Bourgogne wine.
Several compounds such as Propylene Glycol and Methyl salicylate in Bordeaux wines and 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl-2(1H)-Naphthalenone in Bourgogne wines were detected as characteristic compounds for only one of the two types of wine.
The TICC's of EI and PI measurements for 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl-2(1H)-Naphthalenone is shown in Fig.5. Although the peak corresponding to 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl-2(1H)-Naphthalenone was detected as a shoulder peak beside of a huge peak, it was clearly detected using the chromatographic deconvolution functionality of msFineAnalysis AI. The mass spectra of 3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl-2(1H)-Naphthalenone measured by EI and PI is shown Fig. 6.

In total 14 compounds were found as characteristic compounds from only Bordeaux and only Bourgogne wine in this study. It is suggested that those compounds contribute the characteristic aroma and taste for both wines.

Conclusion

In this MSTips, we introduced an example of difference analysis by using msFineAnalysis AI concerning aroma components in wines from different production areas.
By using msFineAnalysis AI, qualitative analysis for GC-TOFMS is expected to be more accurate and efficient.

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Applications note

MS
MSTips No. 328
GC-TOFMS Application

Differential Analysis Function in msFineAnalysis Ver 3 (2): Analysis of Coffee Bean Aroma Component using Headspace-GC-TOFMS

Related product: Mass spectrometer (MS)

Introduction

As the performance of mass spectrometers has improved, the demand for differential analysis of trace components in materials has increased. To address this trend, we have added a new differential analysis function to msFineAnalysis, our automated qualitative analysis software specifically designed for GC-HRTOFMS data. In this work we used msFineAnalysis to compare coffee bean aromas by using headspace (HS)-GC-MS. Additionally, the analysis results were consistent with the characteristics of the production area for the coffee beans.

Analysis detail

Two commercially available coffee beans (A: from Guatemala, B: from Brazil) were used as samples. The measurements were performed with the steps below.

1) Put 1g of coffee beans in a 22mL HS vial, pour 15mL of boiling water to 100°C, and seal.
2) Cool the sample vial to room temperature, collect 10mL of supernatant liquid, and add 2μL of the internal standard (p-Bromofluorobenzene).
3) Separate the above into 2mL each for 5 HS vials and seal.

GC/EI measurements were performed for n=5 in order to conduct statistical analysis of the sample differences. The msFineAnalysis differential analysis conditions involved using p-value (an index: the smaller the p-value, the higher the statistical reproducibility) ≦5% and fold change (intensity ratio between samples) ≧1.5. Table 1 shows the measurements condition details.

Table 1. Measurement and analysis conditions

HS-GC-MS	
Headspace sampler	MS62070STRAP (JEOL)
Mode	Trap
Sample heating	60℃, 15min
Gas Chromatograph	7890A GC (Agilent Technologies, Inc.)
Mode	Split mode (30:1)
Column	ZB-WAX (Phenomenex Inc) 30m x 0.18mm, 0.18μm
Oven Temperature	40°C(3min)-30°C/min -250°C(10min)
Carrier flow	He:1.0mL/min

TOFMS	JMS-T200GC (JEOL)
Ionization	EI+:70eV, 300μA FI+:-10kV, 8mA, Carbotec-5μm (CarboTech)
Mass Range	*m/z* 35-600
msFineAnalysis	(JEOL)
Mode	Variance component analysis
Number of data	n=5
p-value	≦5%
Fold change	≧1.5

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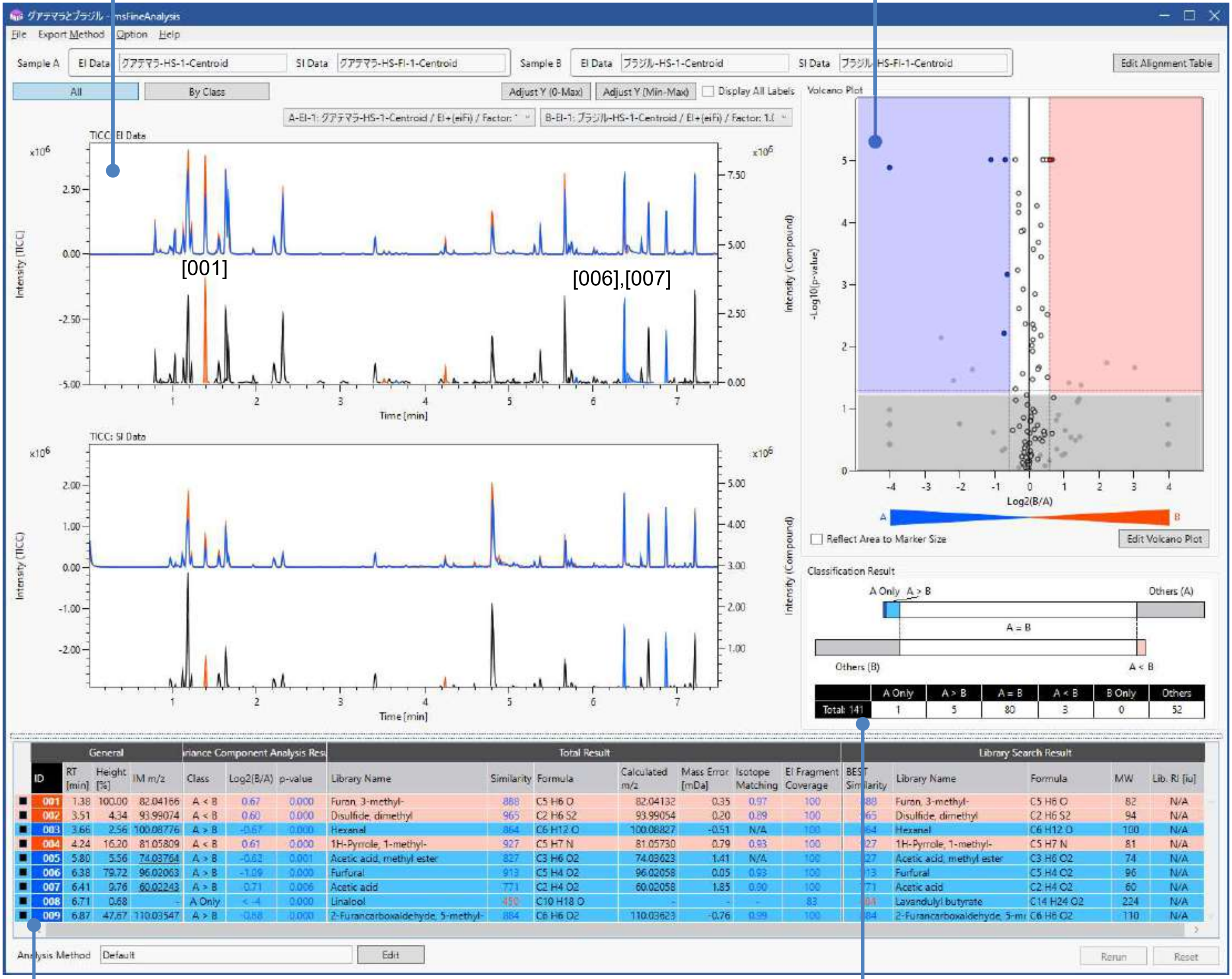
Results

Figure 1 shows a screenshot of the differential analysis results for the coffee samples by using msFineAnalysis. In total, 141 peaks were detected. The breakdown of the differential peaks are: Sample A: 6 peaks that are characteristic of Guatemalan coffee aroma (peak ID[006:]furfural, [007]acetic acid, etc.), Sample B: 3 peaks that are characteristic of Brazilian coffee ([001] metylfuran, etc.), and 52 peaks that did not show a difference between Sample A and B. Additionally, there were 88 peaks that were judged to have no statistical reproducibility (gray in volcano plot, “other” in classification results).

From Sample A, the Guatemalan coffee, hexanal (aroma of grass), methyl acetate (fruit-like aroma), acetic acid, linalool (citrus/floral aroma) were strongly detected. These results are consistent with the freshness and crispness that are characteristics of the coffee beans from Guatemala. From Sample B, the Brazilian coffee, methylfuran (chocolate-like aroma), dimethyl disulfide (garlic-like aroma) were detected, which is consistent with the richness that is characteristic of coffee beans from Brazil.

Chromatogram
Top: GC/EI(TICC/compound peak), Bottom: GI/FI
The color of the compound peak reflects the results of difference judgment
Blue = Strong with Sample A (Guatemala’s)
Red = Strong with Sample B (Brazil’s)
Yellow = No intensity difference (<2 times)

Volcano plot
X axis: Log2(fold change) Y axis:-Log10(p-value)
Blue = Strong with Sample A (Guatemala’s)
Red = Strong with Sample B (Brazil’s)
White = No intensity difference (<2 times)
Gray = Low reproducibility


グアテマラとブラジル - msFineAnalysis
File
Export Method
Option
Help
Edit Alignment Table
All
By Class
Adjust Y (0-Max)
Adjust Y (Min-Max)
☐ Display All Labels
A-EI-1: グアテマラ-HS-1-Centroid / EI+(eiFi) / Factor:
B-EI-1: ブラジル-HS-1-Centroid / EI+(eiFi) / Factor: 1.(
TICC: EI Data
x10^6
2.50
0.00
-2.50
-5.00
7.50
5.00
2.50
0.00
Intensity (TICC)
Intensity (Compound)
[001]
[006],[007]
1
2
3
4
5
6
7
Time [min]
TICC: SI Data
2.00
1.00
0.00
-1.00
-2.00
5.00
4.00
3.00
2.00
1.00
Volcano Plot
-Log10(p-value)
Log2(B/A)
-4
-3
-2
-1
0
1
2
3
4
A
B
☐ Reflect Area to Marker Size
Edit Volcano Plot
Classification Result
A Only
A > B
Others (A)
A = B
Others (B)
A < B
Edit
Rerun
Reset

Sample A: EI Data グアテマラ-HS-1-Centroid; SI Data グアテマラ-HS-FI-1-Centroid
Sample B: EI Data ブラジル-HS-1-Centroid; SI Data ブラジル-HS-FI-1-Centroid

	A Only	A > B	A = B	A < B	B Only	Others
Total: 141	1	5	80	3	0	52

	General				riance Component Analysis Res			Total Result							Library Search Result				
	ID	RT [min]	Height [%]	IM m/z	Class	Log2(B/A)	p-value	Library Name	Similarity	Formula	Calculated m/z	Mass Error [mDa]	Isotope Matching	EI Fragment Coverage	BEST Similarity	Library Name	Formula	MW	Lib. RI [iu]
■	001	1.38	100.00	82.04166	A < B	0.67	0.000	Furan, 3-methyl-	888	C5 H6 O	82.04132	0.35	0.97	100	88	Furan, 3-methyl-	C5 H6 O	82	N/A
■	002	3.51	4.34	93.99074	A < B	0.60	0.000	Disulfide, dimethyl	965	C2 H6 S2	93.99054	0.20	0.89	100	65	Disulfide, dimethyl	C2 H6 S2	94	N/A
■	003	3.66	2.56	100.08776	A > B	-0.67	0.000	Hexanal	864	C6 H12 O	100.08827	-0.51	N/A	100	64	Hexanal	C6 H12 O	100	N/A
■	004	4.24	16.20	81.05809	A < B	0.61	0.000	1H-Pyrrole, 1-methyl-	927	C5 H7 N	81.05730	0.79	0.93	100	27	1H-Pyrrole, 1-methyl-	C5 H7 N	81	N/A
■	005	5.80	5.56	74.03764	A > B	-0.62	0.001	Acetic acid, methyl ester	827	C3 H6 O2	74.03623	1.41	N/A	100	27	Acetic acid, methyl ester	C3 H6 O2	74	N/A
■	006	6.38	79.72	96.02063	A > B	-1.09	0.000	Furfural	913	C5 H4 O2	96.02058	0.05	0.93	100	13	Furfural	C5 H4 O2	96	N/A
■	007	6.41	9.76	60.02243	A > B	-0.71	0.006	Acetic acid	771	C2 H4 O2	60.02058	1.85	0.90	100	71	Acetic acid	C2 H4 O2	60	N/A
■	008	6.71	0.68	-	A Only	< -4	0.000	Linalool	450	C10 H18 O	-	-	-	83	[illegible]	Lavandulyl butyrate	C14 H24 O2	224	N/A
■	009	6.87	47.67	110.03547	A > B	-0.88	0.000	2-Furancarboxaldehyde, 5-methyl-	884	C6 H6 O2	110.03623	-0.76	0.99	100	84	2-Furancarboxaldehyde, 5-m	C6 H6 O2	110	N/A

Analysis Method: Default

Peak List
The color reflects the results of difference judgement
Blue = Strong with sample A(Guatemala’s)
Red = Strong with sample B(Brazil’s)
Note: Only peak of difference are shown.

Classification Result
The ones with high reproducibility are classified into
A only, A>B, A=B, A<B, B only.
The ones with low reproducibility are classified into others.

Figure 1. Screenshot of msFineAnalysis

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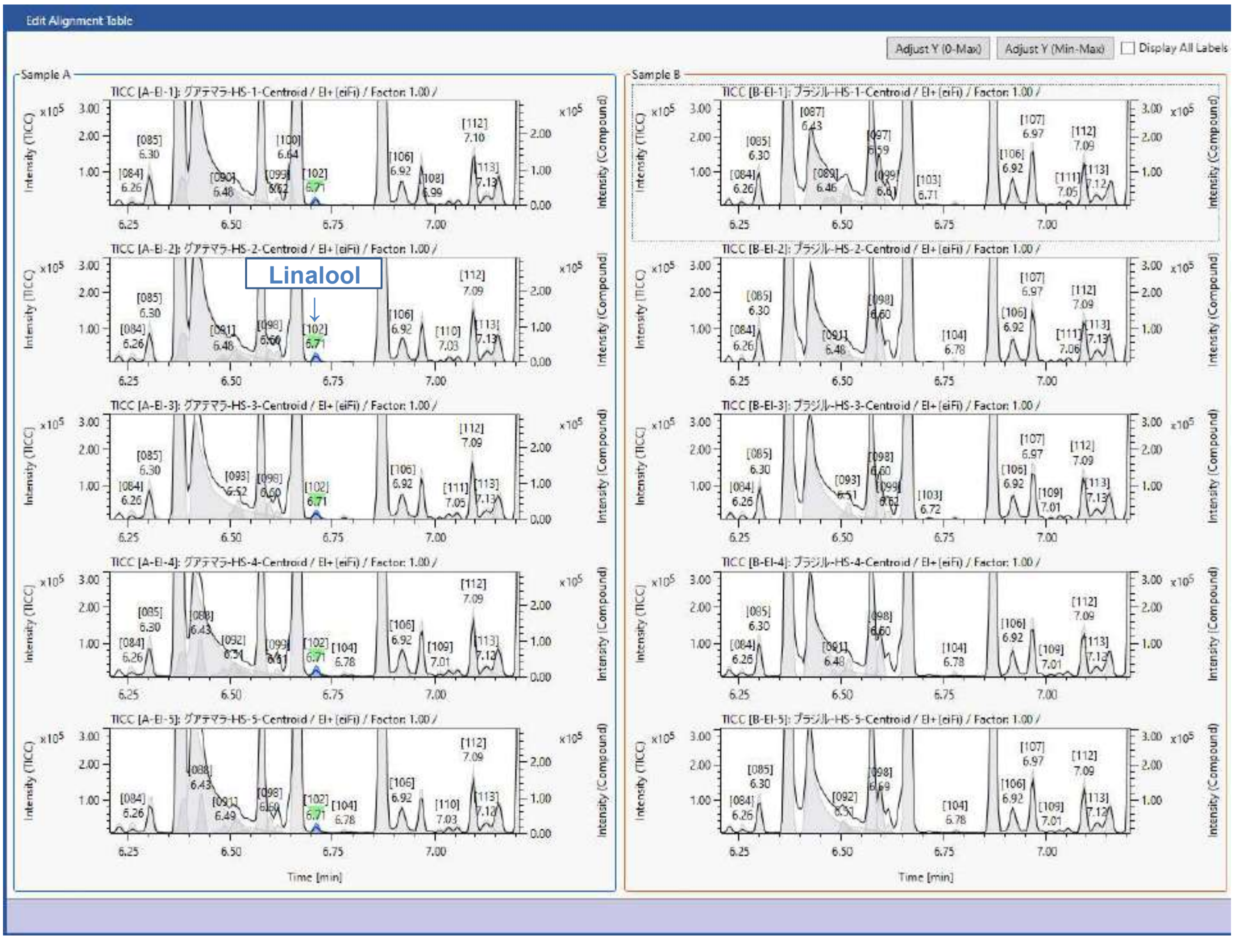
Figure 2 shows a screenshot of the data alignment (identity determination) of msFineAnalysis. Linalool is represented by a very minor peak with an intensity ratio of 0.68% to the maximum peak, and is likely to be missed by manual analysis. By using peak deconvolution detection, msFineAnalysis allows for the detection of minor peaks that are not obvious in the TICC . Furthermore, the statistical analysis of multiple measurements results in highly reliable qualitative analysis results by removing peaks from the analysis list that are not statistically reproducible.


Sample A Guatemalan Coffee

Sample B Brazilian Coffee
Edit Alignment Table
Adjust Y (0-Max)
Adjust Y (Min-Max)
☐ Display All Labels
Sample A
Sample B
TICC [A-EI-1]: グアテマラ-HS-1-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [A-EI-2]: グアテマラ-HS-2-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [A-EI-3]: グアテマラ-HS-3-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [A-EI-4]: グアテマラ-HS-4-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [A-EI-5]: グアテマラ-HS-5-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [B-EI-1]: ブラジル-HS-1-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [B-EI-2]: ブラジル-HS-2-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [B-EI-3]: ブラジル-HS-3-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [B-EI-4]: ブラジル-HS-4-Centroid / EI+ (eiFi) / Factor: 1.00 /
TICC [B-EI-5]: ブラジル-HS-5-Centroid / EI+ (eiFi) / Factor: 1.00 /
Linalool
Intensity (TICC)
Intensity (Compound)
x10^5
3.00
2.00
1.00
0.00
6.25
6.50
6.75
7.00
Time [min]
[084] 6.26
[085] 6.30
[102] 6.71
[106] 6.92
[107] 6.97
[112] 7.09
[113] 7.13

Figure 2. Screenshot of alignment window

Summary

The differential analysis function of msFineAnalysis Ver3 enabled us to easily identify coffee bean aroma components that are consistent with the characteristic of the production region (Guatemalan versus Brazilian). As a result, msFineAnalysis offers sophisticated capabilities such as peak deconvolution and statistical analysis to effectively differentiate samples from each by using trace components such as an aromas for sample differentiation.

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Integrated Analysis of Fatty Acid Methyl Esters using msFineAnalysis Version 2
- Molecular Ion Detection by FI -

Product: Mass spectrometer (MS)

[Introduction]

Electron ionization (EI) is a hard ionization method that is commonly used with gas chromatography mass spectrometry (GC-MS). The mass spectral fragmentation patterns produced by EI are used for library database searches to identify compounds. Conversely, soft ionization methods like field ionization (FI) tend to produce clear molecular ions with minimal fragmentation. When high-resolution MS is used with these ionization techniques, the accurate masses for the fragment ions produced by EI and the molecular ions produced by soft ionization provide an additional dimension of information for the analytes. Combining the exact mass information with the results of conventional library search can enhance the accuracy of identification compared to the use of library search alone.

Fatty acid methyl esters (FAMEs) are crucial for determining the fat content in food. Being environmentally friendly, they are also increasingly used as bio-diesel fuels. Many of the FAMEs are unsaturated with double bonds in the alkyl chains. As the number of double bonds increases (more unsaturation), the EI measurements tend to lack molecular ions. In this work, we measured a standard sample containing multiple FAMEs using EI and FI to detect their molecular ions. The resulting data was further examined by using msFineAnalysis to produce an integrated report for these compounds in which the library database search was combined with the molecular ion exact mass analysis to produce a qualitative identification of these compounds.

[Experimental]

A commercial 37-component FAME standard mixture (Restek, 200-600 ng/μL) was used as a sample. Table 1 shows the measurement conditions used for the GC/EI and GC/FI analyses.

[Results and Discussion]

Figure 1 shows the TICC for the GC/EI and GC/FI measurements. While the sample contains 37 components, there were only 36 peaks observed in each chromatogram. The *cis*-4,7,10,13,16,19-docosahexaenoic acid methyl ester ($C_{23}H_{34}O_2$) and the heneicosanoic acid methyl ester ($C_{22}H_{44}O_2$) coelute with exactly the same retention time (RT) at 38.8 min. However, the FI mass spectrum for this peak showed the molecular ions for each component (Figure 2). Because the JMS-T200GC is always measuring high-resolution mass spectra, these components, which are not quite separated in the chromatogram, can be identified by mass separation.

Table 1. Measurement conditions

[GC Conditions]	
GC system:	7890A (Agilent Technologies)
Column:	DB-5msUI, 30 m x 0.25 mm, 0.25 μm
Oven temperature:	50°C (1 min) → 10°C/min → 140°C → 3°C/min → 260°C (5 min)
Injection mode:	Split mode (50:1)
[TOFMS Conditions]	
MS system:	JMS-T200GC (JEOL Ltd.)
Ion source:	EI/FI combined ion source
Ionization:	EI+, 70 eV, 300 μA FI+, -10 kV, 50 mA (slope mode)
Mass range:	*m/z* 35-600

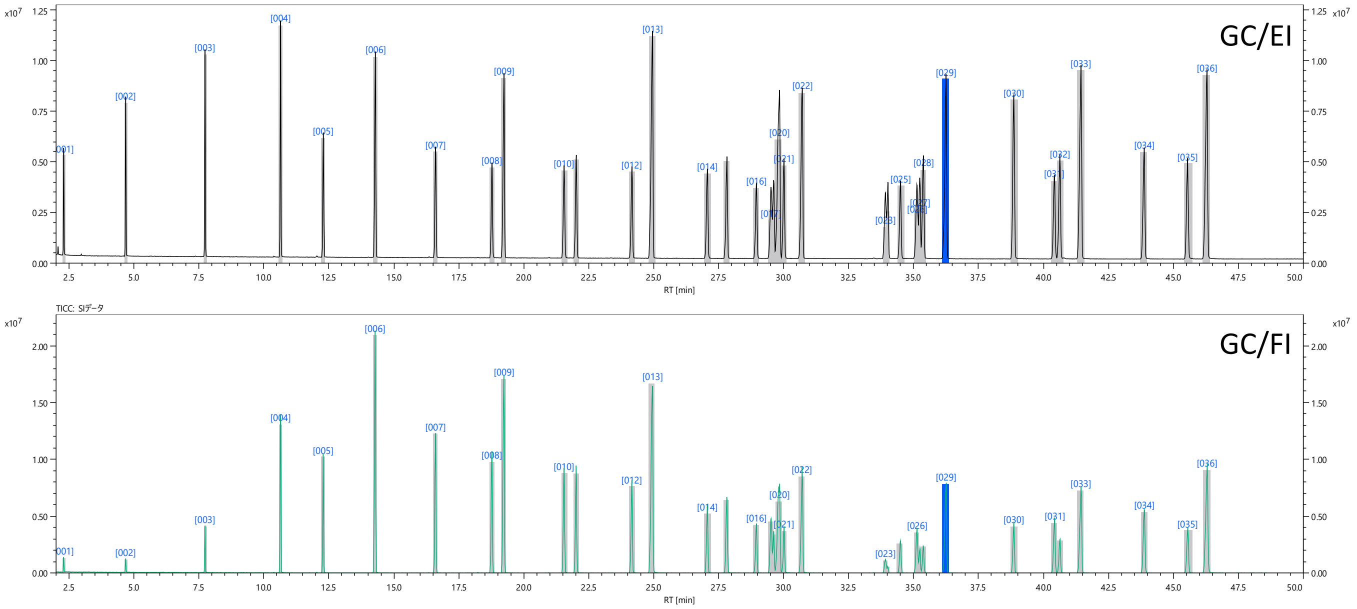

Figure 1. GC/EI and GC/FI total ion current chromatograms for the 37 FAME mixture

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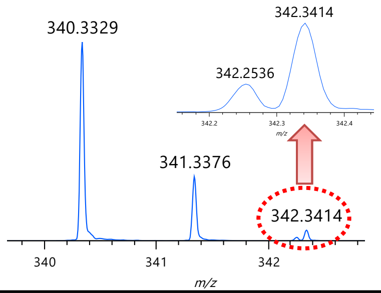

m/z	Formula	Error [mDa]	DBE
340.3329	$C_{22}H_{44}O_2$	-0.7	1.0
342.2536	$C_{23}H_{34}O_2$	-1.8	7.0

Figure 2. FI mass spectrum (enlarged) at RT 38.8 min and exact mass analysis results

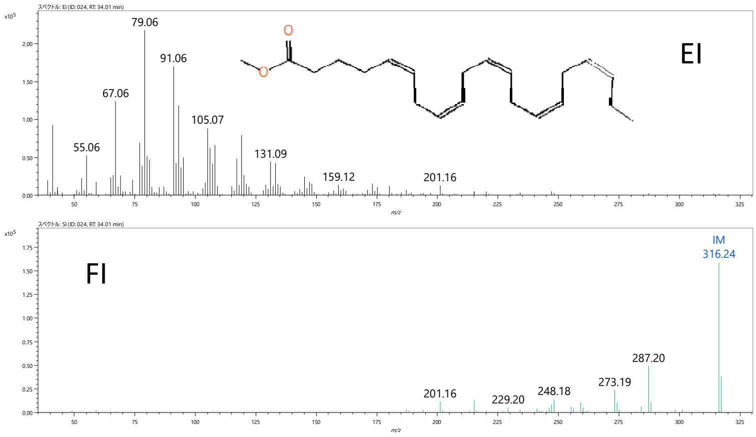

Figure 3. EI and FI mass spectra of 5, 8, 11, 14, 17-eicosapentaenoic acid methyl ester (all-Z)-

The FI mass spectra show molecular ions for all 37 FAMEs in the mixture. Additionally, these molecular ions are the base peak in each FI mass spectrum except for the 15-tetracosenoic acid methyl ester (Z)-, which is detected at a relative intensity of >80%. All of these results demonstrate that FI ionizes FAMEs softly and efficiently. As an example, Figure 3 shows the EI and FI mass spectra for 5,8,11,14,17-eicosapentaenoic acid methyl ester (all-Z)-, which has 5 double bonds and an alkyl group. In this example, the molecular ion was not observed in the EI mass spectrum, but the molecular ion is the base peak in the FI mass spectrum. Figure 4 shows the FI mass spectra and chemical formulas for 6 components that all have a carbon number of 20 (minus the ester bond) and have 0 to 5 double bonds. Lastly, Table 2 shows the integrated analysis report generated by msFineAnalysis. In each case, the FI molecular ion accurate masses were automatically used to determine the molecular formula for each component in the FAMES mixture to help identify the correct match from the EI library database search.

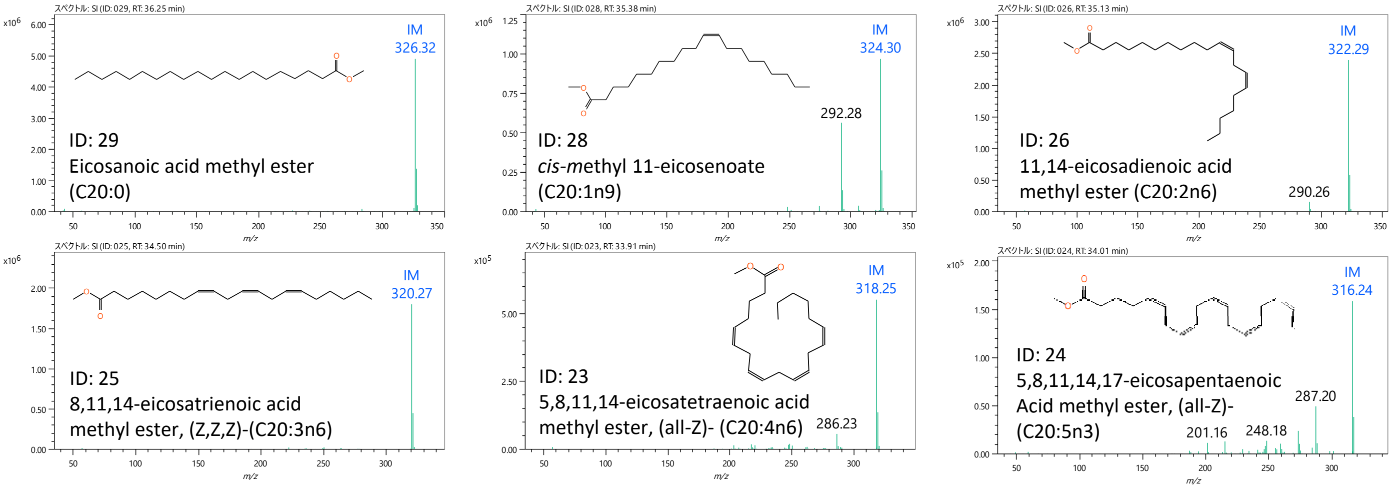

Figure 4. FI mass spectra for the C20 FAMEs.

[Conclusions]

The msFineAnalysis integrated analysis method produces highly accurate qualitative analysis results for the FAMEs by combining the library search results and molecular formula estimation. This combination of using GC/EI and GC/FI measurements together for qualitative analysis is particularly important for FAMEs as these types of compounds do not produce molecular ions for EI, making it difficult to use database searches alone for identification.

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Table 2. Integrated qualitative analysis results report using msFineAnalysis

General					Total Result						Library Search Result				
ID	RT [min]	Area	Area [%]	IM m/z	Library Name	CAS#	Similarity	Formula	Calculated m/z	Mass Error [mDa]	BEST Similarity	Library Name	CAS#	Formula	MW
001	2.29	7,802,029	14.13	102.06850	Butanoic acid, methyl ester	623-42-7	902	$C_{5}H_{10}O_{2}$	102.06753	0.97	902	Butanoic acid, methyl ester	623-42-7	$C_{5}H_{10}O_{2}$	102
002	4.68	12,509,365	22.65	130.09965	Hexanoic acid, methyl ester	106-70-7	954	$C_{7}H_{14}O_{2}$	130.09883	0.82	954	Hexanoic acid, methyl ester	106-70-7	$C_{7}H_{14}O_{2}$	130
003	7.74	17,836,754	32.30	158.13091	Octanoic acid, methyl ester	111-11-5	921	$C_{9}H_{18}O_{2}$	158.13013	0.78	921	Octanoic acid, methyl ester	111-11-5	$C_{9}H_{18}O_{2}$	158
004	10.64	22,872,875	41.42	186.16241	Decanoic acid, methyl ester	110-42-9	951	$C_{11}H_{22}O_{2}$	186.16143	0.98	951	Decanoic acid, methyl ester	110-42-9	$C_{11}H_{22}O_{2}$	186
005	12.28	13,873,023	25.12	200.17785	Undecanoic acid, methyl ester	1731-86-8	947	$C_{12}H_{24}O_{2}$	200.17708	0.77	947	Undecanoic acid, methyl ester	1731-86-8	$C_{12}H_{24}O_{2}$	200
006	14.29	28,637,881	51.86	214.19378	Dodecanoic acid, methyl ester	111-82-0	924	$C_{13}H_{26}O_{2}$	214.19273	1.05	924	Dodecanoic acid, methyl ester	111-82-0	$C_{13}H_{26}O_{2}$	214
007	16.61	16,639,305	30.13	228.20907	Tridecanoic acid, methyl ester	1731-88-0	962	$C_{14}H_{28}O_{2}$	228.20838	0.68	962	Tridecanoic acid, methyl ester	1731-88-0	$C_{14}H_{28}O_{2}$	228
008	18.78	15,928,565	28.84	240.20818	Methyl myristoleate	56219-06-8	951	$C_{15}H_{28}O_{2}$	240.20838	-0.20	951	Methyl myristoleate	56219-06-8	$C_{15}H_{28}O_{2}$	240
009	19.24	33,711,996	61.05	242.22503	Methyl tetradecanoate	124-10-7	956	$C_{15}H_{30}O_{2}$	242.22403	1.00	956	Methyl tetradecanoate	124-10-7	$C_{15}H_{30}O_{2}$	242
010	21.55	16,820,354	30.46	254.22386	Methyl (Z)-10-pentadecenoate	-	928	$C_{16}H_{30}O_{2}$	254.22403	-0.18	928	Methyl (Z)-10-pentadecenoate	-	$C_{16}H_{30}O_{2}$	254
011	22.02	18,711,161	33.88	256.24022	Pentadecanoic acid, methyl ester	7132-64-1	949	$C_{16}H_{32}O_{2}$	256.23968	0.54	949	Pentadecanoic acid, methyl ester	7132-64-1	$C_{16}H_{32}O_{2}$	256
012	24.16	17,805,851	32.24	268.23913	9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	942	$C_{17}H_{32}O_{2}$	268.23968	-0.55	942	9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	$C_{17}H_{32}O_{2}$	268
013	24.95	55,221,475	100.00	270.25537	Hexadecanoic acid, methyl ester	112-39-0	946	$C_{17}H_{34}O_{2}$	270.25533	0.04	946	Hexadecanoic acid, methyl ester	112-39-0	$C_{17}H_{34}O_{2}$	270
014	27.07	18,586,681	33.66	282.25402	cis-10-Heptadecenoic acid, methyl ester	-	943	$C_{18}H_{34}O_{2}$	282.25533	-1.32	943	cis-10-Heptadecenoic acid, methyl ester	-	$C_{18}H_{34}O_{2}$	282
015	27.82	20,566,515	37.24	284.27051	Heptadecanoic acid, methyl ester	1731-92-6	933	$C_{18}H_{36}O_{2}$	284.27098	-0.47	933	Heptadecanoic acid, methyl ester	1731-92-6	$C_{18}H_{36}O_{2}$	284
016	28.96	15,344,971	27.79	292.23830	Methyl γ-linolenate	16326-32-2	945	$C_{19}H_{32}O_{2}$	292.23968	-1.38	945	Methyl γ-linolenate	16326-32-2	$C_{19}H_{32}O_{2}$	292
017	29.52	8,858,244	16.04	294.25444	9,12-Octadecadienoic acid, methyl ester, (E,E)-	2566-97-4	835	$C_{19}H_{34}O_{2}$	294.25533	-0.89	877	11,14-Octadecadienoic acid, methyl ester	56554-61-1	$C_{19}H_{34}O_{2}$	294
018	29.62	8,877,607	16.08	292.23851	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	301-00-8	918	$C_{19}H_{32}O_{2}$	292.23968	-1.17	918	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	301-00-8	$C_{19}H_{32}O_{2}$	292
019	29.79	18,365,132	33.26	294.25465	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	866	$C_{19}H_{34}O_{2}$	294.25533	-0.68	866	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	$C_{19}H_{34}O_{2}$	294
020	29.84	32,367,534	58.61	296.26984	9-Octadecenoic acid (Z)-, methyl ester	112-62-9	894	$C_{19}H_{36}O_{2}$	296.27098	-1.14	894	9-Octadecenoic acid (Z)-, methyl ester	112-62-9	$C_{19}H_{36}O_{2}$	296
021	30.02	19,095,805	34.58	296.27036	9-Octadecenoic acid, methyl ester, (E)-	1937-62-8	927	$C_{19}H_{36}O_{2}$	296.27098	-0.62	941	trans-13-Octadecenoic acid, methyl ester	-	$C_{19}H_{36}O_{2}$	296
022	30.72	41,648,466	75.42	298.28652	Methyl stearate	112-61-8	939	$C_{19}H_{38}O_{2}$	298.28663	-0.11	939	Methyl stearate	112-61-8	$C_{19}H_{38}O_{2}$	298
023	33.91	6,037,723	10.93	318.25265	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	2566-89-4	909	$C_{21}H_{34}O_{2}$	318.25533	-2.68	909	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	2566-89-4	$C_{21}H_{34}O_{2}$	318
024	34.01	6,574,800	11.91	316.23636	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	2734-47-6	945	$C_{21}H_{32}O_{2}$	316.23968	-3.32	945	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	2734-47-6	$C_{21}H_{32}O_{2}$	316
025	34.50	16,559,406	29.99	320.26864	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	21061-10-9	937	$C_{21}H_{36}O_{2}$	320.27098	-2.34	937	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	21061-10-9	$C_{21}H_{36}O_{2}$	320
026	35.13	10,403,767	18.84	322.28535	11,14-Eicosadienoic acid, methyl ester	2463-02-7	820	$C_{21}H_{38}O_{2}$	322.28663	-1.28	902	cis-11,14-Eicosadienoic acid, methyl ester	-	$C_{21}H_{38}O_{2}$	322
027	35.25	9,642,111	17.46	320.26997	11,14,17-Eicosatrienoic acid, methyl ester	55682-88-7	861	$C_{21}H_{36}O_{2}$	320.27098	-1.01	861	11,14,17-Eicosatrienoic acid, methyl ester	55682-88-7	$C_{21}H_{36}O_{2}$	320
028	35.38	18,118,358	32.81	324.30086	cis-Methyl 11-eicosenoate	2390-09-2	948	$C_{21}H_{40}O_{2}$	324.30228	-1.42	948	cis-Methyl 11-eicosenoate	2390-09-2	$C_{21}H_{40}O_{2}$	324
029	36.25	45,036,437	81.56	326.31784	Eicosanoic acid, methyl ester	1120-28-1	895	$C_{21}H_{42}O_{2}$	326.31793	-0.10	936	Methyl 18-methylnonadecanoate	-	$C_{21}H_{42}O_{2}$	326
030	38.86	36,312,501	65.76	340.33291	Heneicosanoic acid, methyl ester	6064-90-0	781	$C_{22}H_{44}O_{2}$	340.33358	-0.67	781	Heneicosanoic acid, methyl ester	6064-90-0	$C_{22}H_{44}O_{2}$	340
031	40.42	18,929,601	34.28	350.31571	cis-13,16-Docasadienoic acid, methyl ester	-	947	$C_{23}H_{42}O_{2}$	350.31793	-2.22	947	cis-13,16-Docasadienoic acid, methyl ester	-	$C_{23}H_{42}O_{2}$	350
032	40.63	22,206,388	40.21	352.33120	13-Docosenoic acid, methyl ester, (Z)-	1120-34-9	881	$C_{23}H_{44}O_{2}$	352.33358	-2.38	912	Methyl 11-docosenoate	-	$C_{23}H_{44}O_{2}$	352
033	41.44	48,576,846	87.97	354.34851	Docosanoic acid, methyl ester	929-77-1	940	$C_{23}H_{46}O_{2}$	354.34923	-0.73	940	Docosanoic acid, methyl ester	929-77-1	$C_{23}H_{46}O_{2}$	354
034	43.88	25,785,457	46.69	368.36379	Tricosanoic acid, methyl ester	2433-97-8	947	$C_{24}H_{48}O_{2}$	368.36488	-1.09	947	Tricosanoic acid, methyl ester	2433-97-8	$C_{24}H_{48}O_{2}$	368
035	45.55	23,274,202	42.15	380.36207	15-Tetracosenoic acid, methyl ester, (Z)-	2733-88-2	871	$C_{25}H_{48}O_{2}$	380.36488	-2.81	876	15-Tetracosenoic acid, methyl ester	56554-33-7	$C_{25}H_{48}O_{2}$	380
036	46.29	51,225,970	92.76	382.37968	Tetracosanoic acid, methyl ester	2442-49-1	922	$C_{25}H_{50}O_{2}$	382.38053	-0.85	922	Tetracosanoic acid, methyl ester	2442-49-1	$C_{25}H_{50}O_{2}$	382

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Integrated Analysis of Coffee Aroma by using a Headspace GC-HRMS

- Developing an Integrated Analysis Technique using Data Acquired by GC/EI and GC/Soft Ionization -

Product: Mass spectrometer (MS)

[Introduction]

Electron ionization (EI) is a hard ionization method that is commonly used with gas chromatography mass spectrometry (GC-MS). The mass spectral fragmentation patterns produced by EI are used for library database searches to identify compounds. Conversely, soft ionization methods like field ionization (FI) tend to produce clear molecular ions with minimal fragmentation. When high-resolution MS is used with these ionization techniques, the accurate masses for the fragment ions produced by EI and the molecular ions produced by soft ionization provide an additional dimension of information for the analytes. Combining the exact mass information with the results of conventional library search can enhance the accuracy of identification compared to the use of library search alone. In this work, we introduce the msFineAnalysis software and use it to automatically combine data acquired by GC/EI and GC/soft ionization for the qualitative analysis of coffee headspace.

[Experimental]

A commercial coffee was prepared as follows:

1) One gram of coffee beans was loaded into a 22 mL vial, 15 mL of boiling water was added, and the vial was sealed.
2) After the sample was cooled to room temperature, 10 mL of the supernatant was loaded into another vial, and 2 µL of an internal reference (p-Bromofluorobenzene) solution was added to the sample.
3) Finally, 2 mL of the above solution was transferred to the vail for the headspace sampler and sealed in a vial that was then used as a sample.

Table 1 shows the measurement conditions used for the headspace/GC-TOFMS system.

Table 1. Measurement Conditions

[Conditions of headspace sampler]

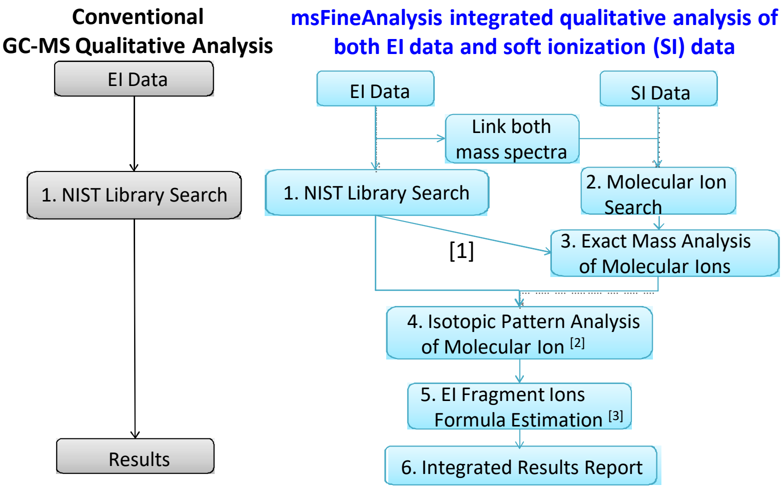
System	MS-62070STRAP (JEOL)
Mode	Trap mode
Extract	3 times
Heating condition	60°C, 15 min

[GC-TOFMS Conditions]

System	JMS-T200GC (JEOL)
Ionization mode	EI+: 70 eV, 300 µA FI+: -10 kV, 8mA (Carbotec 5 µm)
GC column	ZB-WAX, 30 m x 0.18 mm, 0.18 mm
Oven temp.	40°C (3 min) → 30°C/min → 250°C (10 min)
Inlet temperature	250°C
Inlet mode	Split 30:1

[Results and Discussion]

Figure 1 shows the operational flow chart for the integrated analysis steps used for the JEOL msFineAnalysis software (chart on the right). First, the data is acquired by using both EI and soft ionization (SI), and all peaks and associated mass spectra are detected in the chromatograms. Afterwards, the mass spectra produced by these ionization methods are linked using their retention times, and these linked mass spectra are recorded as single components. Next, the EI mass spectrum is used for the library database search (1), and the SI mass spectrum is used to identify the analyte molecular ion (2). Afterwards, the molecular ion is used for exact mass analysis to determine possible elemental compositions, and these candidate formulas are then filtered by using the EI library search results (3). Next, the molecular ion is subjected to isotopic pattern analysis to help further limit the candidate formulas (4). Each candidate formula is then used as a search constraint for the exact mass analysis of the EI fragment ions (5). If the molecular ion formula candidate is incorrect, the EI fragment ions will not result in many (if any) compositional formulas, thus indicating that the molecular ion formula is not a good candidate for that particular analyte. These results are then output as an integrated qualitative report (6).


Conventional
GC-MS Qualitative Analysis
EI Data
1. NIST Library Search
Results
msFineAnalysis integrated qualitative analysis of both EI data and soft ionization (SI) data
EI Data
SI Data
Link both mass spectra
1. NIST Library Search
2. Molecular Ion Search
[1]
3. Exact Mass Analysis of Molecular Ions
4. Isotopic Pattern Analysis of Molecular Ion [2]
5. EI Fragment Ions Formula Estimation [3]
6. Integrated Results Report

[1] Use the library search results as the condition for estimating molecular ion
[2] Molecular ions can be selected from EI data
[3] Use the results of estimated molecular ion composition as conditions for estimating fragment ion compositions

Figure 1. Qualitative Analysis Flow

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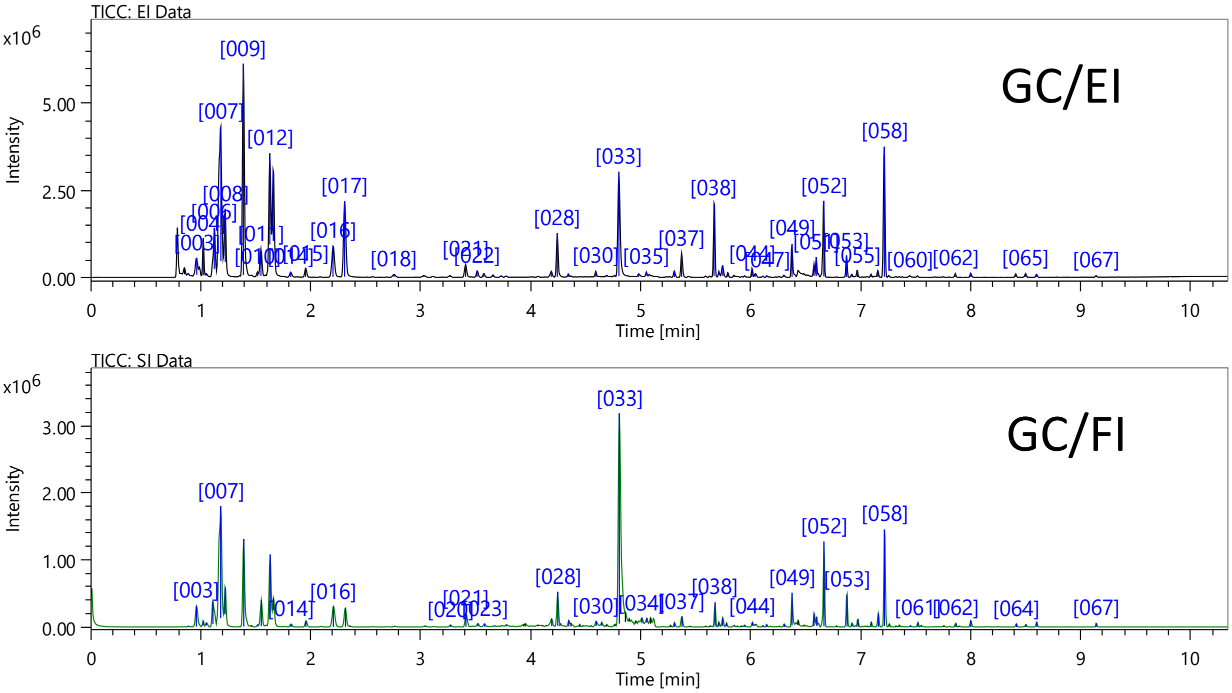
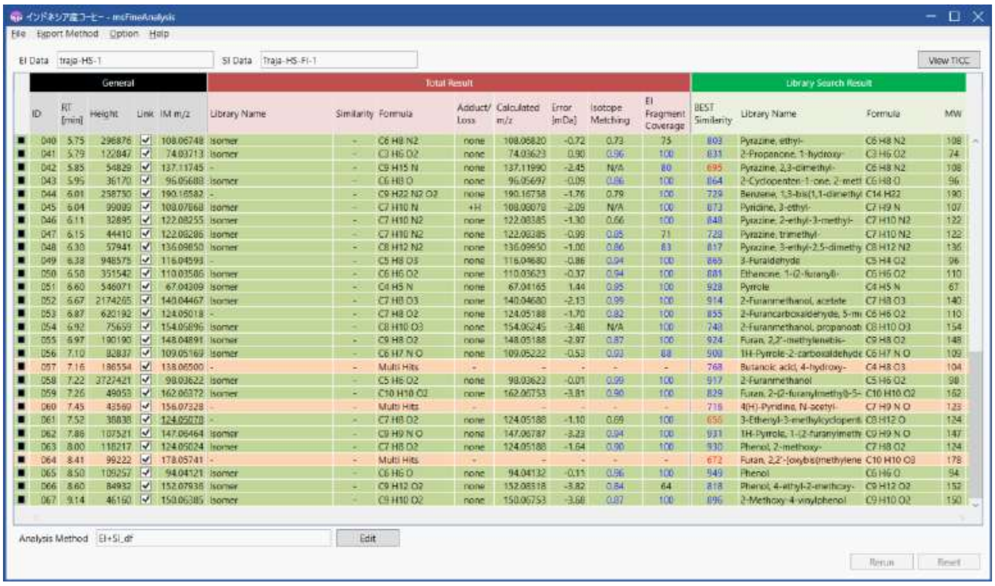


Figure 2. TIC chromatograms of coffee aroma acquired by a HS/GC/TOFMS



EI Data traja-HS-1 SI Data Traja-HS-FI-1

	General					Total Result										Library Search Result			
	ID	RT [min]	Height	Link	IM m/z	Library Name	Similarity	Formula	Adduct/ Loss	Calculated m/z	Error [mDa]	Isotope Matching	EI Fragment Coverage	BEST Similarity	Library Name	Formula	MW
■	040	5.75	296876	☑	108.06748	Isomer	-	C6 H8 N2	none	108.06820	-0.72	0.73	75	803	Pyrazine, ethyl-	C6 H8 N2	108
■	041	5.79	122847	☑	74.03713	Isomer	-	C3 H6 O2	none	74.03623	0.90	0.96	100	831	2-Propanone, 1-hydroxy-	C3 H6 O2	74
■	042	5.85	54829	☑	137.11745	-	-	C9 H15 N	none	137.11990	-2.45	N/A	80	695	Pyrazine, 2,3-dimethyl-	C6 H8 N2	108
■	043	5.95	36170	☑	96.05688	Isomer	-	C6 H8 O	none	96.05697	-0.09	0.86	100	864	2-Cyclopenten-1-one, 2-met	C6 H8 O	96
■	044	6.01	258750	☑	190.16582	-	-	C9 H22 N2 O2	none	190.16738	-1.76	0.79	100	729	Benzene, 1,3-bis(1,1-dimethyl	C14 H22	190
■	045	6.04	99089	☑	108.07868	Isomer	-	C7 H10 N	+H	108.08078	-2.09	N/A	100	873	Pyridine, 3-ethyl-	C7 H9 N	107
■	046	6.11	32895	☑	122.08255	Isomer	-	C7 H10 N2	none	122.08385	-1.30	0.66	100	848	Pyrazine, 2-ethyl-3-methyl-	C7 H10 N2	122
■	047	6.15	44410	☑	122.08286	Isomer	-	C7 H10 N2	none	122.08385	-0.99	0.05	71	728	Pyrazine, trimethyl-	C7 H10 N2	122
■	048	6.30	57941	☑	136.09850	Isomer	-	C8 H12 N2	none	136.09950	-1.00	0.86	83	817	Pyrazine, 3-ethyl-2,5-dimethy	C8 H12 N2	136
■	049	6.38	948575	☑	116.04593	-	-	C5 H8 O3	none	116.04680	-0.86	0.94	100	865	3-Furaldehyde	C5 H4 O2	96
■	050	6.58	351542	☑	110.03586	Isomer	-	C6 H6 O2	none	110.03623	-0.37	0.94	100	881	Ethanone, 1-(2-furanyl)-	C6 H6 O2	110
■	051	6.60	546071	☑	67.04309	Isomer	-	C4 H5 N	none	67.04165	1.44	0.95	100	928	Pyrrole	C4 H5 N	67
■	052	6.67	2174265	☑	140.04467	Isomer	-	C7 H8 O3	none	140.04680	-2.13	0.99	100	914	2-Furanmethanol, acetate	C7 H8 O3	140
■	053	6.87	620192	☑	124.05018	-	-	C7 H8 O2	none	124.05188	-1.70	0.82	100	855	2-Furancarboxaldehyde, 5-m	C6 H6 O2	110
■	054	6.92	75659	☑	154.05896	Isomer	-	C8 H10 O3	none	154.06245	-3.48	N/A	100	748	2-Furanmethanol, propanoat	C8 H10 O3	154
■	055	6.97	190190	☑	148.04891	Isomer	-	C9 H8 O2	none	148.05188	-2.97	0.87	100	924	Furan, 2,2'-methylenebis-	C9 H8 O2	148
■	056	7.10	82837	☑	109.05169	Isomer	-	C6 H7 N O	none	109.05222	-0.53	0.93	88	900	1H-Pyrrole-2-carboxaldehyd	C6 H7 N O	109
■	057	7.16	186554	☑	138.06500	-	-	Multi Hits	-	-	-	-	-	768	Butanoic acid, 4-hydroxy-	C4 H8 O3	104
■	058	7.22	3727421	☑	98.03622	Isomer	-	C5 H6 O2	none	98.03623	-0.01	0.99	100	917	2-Furanmethanol	C5 H6 O2	98
■	059	7.26	49053	☑	162.06372	Isomer	-	C10 H10 O2	none	162.06753	-3.81	0.90	100	829	Furan, 2-(2-furanylmethyl)-5-	C10 H10 O2	162
■	060	7.45	43569	☑	156.07328	-	-	Multi Hits	-	-	-	-	-	718	4(H)-Pyridine, N-acetyl-	C7 H9 N O	123
■	061	7.52	38838	☑	124.05070	-	-	C7 H8 O2	none	124.05188	-1.10	0.69	100	656	3-Ethenyl-3-methylcyclopent	C8 H12 O	124
■	062	7.86	107521	☑	147.06464	Isomer	-	C9 H9 N O	none	147.06787	-3.23	0.94	100	931	1H-Pyrrole, 1-(2-furanylmeth	C9 H9 N O	147
■	063	8.00	118217	☑	124.05024	Isomer	-	C7 H8 O2	none	124.05188	-1.64	0.90	100	930	Phenol, 2-methoxy-	C7 H8 O2	124
■	064	8.41	99222	☑	178.05741	-	-	Multi Hits	-	-	-	-	-	672	Furan, 2,2'-[oxybis(methylene	C10 H10 O3	178
■	065	8.50	109257	☑	94.04121	Isomer	-	C6 H6 O	none	94.04132	-0.11	0.96	100	949	Phenol	C6 H6 O	94
■	066	8.60	84932	☑	152.07938	Isomer	-	C9 H12 O2	none	152.08318	-3.82	0.84	64	818	Phenol, 4-ethyl-2-methoxy-	C9 H12 O2	152
■	067	9.14	46160	☑	150.06385	Isomer	-	C9 H10 O2	none	150.06753	-3.68	0.87	100	896	2-Methoxy-4-vinylphenol	C9 H10 O2	150

Analysis Method EI+SI_df Edit

Figure 3. Integrated qualitative analysis results on msFineAnalysis

The msFineAnalysis Auto Analysis function detected 67 components in the GC/EI and GC/FI measurements (Figure 2) that were automatically linked using their retention time. The Auto Analysis function then automatically used the steps in Figure 1 to analyze the linked data, and the results were output as a color-coded table as shown in Figure 3. Each color indicates a level of confidence for the identity of the compound:

Green:	A molecular formula candidate was uniquely identified.
Orange:	Multiple molecular formula candidates were identified.
White:	No significant molecular formula candidates were identified.

The components classified as orange or white can be further reviewed manually to potentially identify a unique candidate formula. In this example, the software was able to automatically determine a unique molecular formula for 63 of the 67 components in the coffee headspace sample.

[Conclusions]

The msFineAnalysis software produces highly accurate qualitative analysis results by automatically combining the EI library search results and soft ionization (SI) molecular formula determinations. Additionally, this software makes it possible to determine molecular formulas for unknown components not registered in library (match factor score: low), which can not be identified by database search alone (Figure 1, left side). The effectiveness of the msFineAnalysis integrated analysis method effectiveness for GC/MS qualitative analysis was demonstrated by automatically determining molecular formulas from exact masses, regardless of the level of match factor score, to limit the candidate formulas.

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Comprehensive Analysis + Unknown Component Analysis of Coffee Samples Using Headspace GC-MS

- Characteristic Component Extraction by Multiple Classification PCA and Component Identification by High Resolution MS -

Product used：Mass Spectrometer (MS)

[General]

Advances in mass spectrometry are enabling analysis of micro samples and unknown components that were not observable before. As the volume of information acquired from mass spectrometry increases, researchers are calling for simple techniques to analyze numerous components observed, and as a result, there is a rise in demand for comprehensive analytical techniques including multiple classification analysis.

In this work, we will introduce a new technique of non-targeted analysis, which combines comprehensive analysis using high resolution GC-MS and unknown component analysis using soft ionization and EI.

[Method]

Table 1 shows the measurement conditions. Four different types of commercial coffee (A: Indonesian; B: Ethiopian; C: Guatemaran; D: Brazilian) were selected as samples. Each sample was measured 5 times (n=5). The samples were prepared as follows:

1) One gram of coffee beans was loaded into a 22 mL headspace (HS) vial, 15 mL of 100℃ water was added, and then the vial was sealed.
2) After the sample had cooled to room temperature, 10 mL of the supernatant was loaded into another HS vial, and 2 μL of an internal reference (p-Bromofluorobenzene) was added to the sample.
3) Finally, 2 mL of the above solution was sealed in a vial and used as a sample.

Table 1. Measurement Conditions

[Headspace Conditions]

System	MS-62070STRAP (JEOL)
Mode	Trap mode
Extract	3 times
Heating condition	60℃, 15min

[GC-TOFMS Conditions]

System	JMS-T200GC (JEOL)
Ionization mode	EI+: 70 eV, 300 μA, FI+: -10kV, 8mA (Carbotec 5μm)
GC column	ZB-WAX (Phenomenex), 30m x 0.18mm, 0.18μm
Oven temp.	40℃ (3min)→30℃/min →250℃(10min)
Inlet temperature	250℃
Inlet mode	Split30:1
He flow	1.0 mL/min (Constant Flow)
m/z range	*m/z* 35-600
Spectrum recording speed	0.3 sec
Software	AnalyzerPro (SpectralWork)

[Results]

Figure 1 shows the TIC chromatograms acquired. Among the components observed in each sample, differences between the components detected at high intensity were visible. However, it will be an extremely lengthy process to manually examine all of the detected components. Also, because analysis of micro components hidden under the TIC baseline is likely to produce different results by different operators, auto analysis software capable of peak detection under the same conditions is more effective in comparing the components between samples. For comprehensive analysis of volatile components in coffee, SpectralWorks AnalyzerPro was used. AnalyzePro initially extracts the components in question from the chromatogram through deconvolution. The program automatically searches the NIST libraries for all mass spectra of the components selected by deconvolution. The results are tabulated, and the resulting data is subjected to PCA and diffusion analysis.

Figure 2 shows the results of PCA. The PCA score plot classified the measured data according to where they were grown. Specifically, the 1st principal component axis separated the Indonesian coffee (A) from those produced elsewhere. Next, a PCA loading plot was created to identify the components that contributed to the positive separation of the 1st principal component, that is, characteristic components of the Indonesian coffee (A). Figure 3 shows a magnified view of the 1st principal component axis on the positive side in the PCA loading plot (area within a red circle).

Four components shown in Figure 3 contributed the most to the positive separation of the 1st principal component. Of these, 3 components were identified through NIST library search. For example, pyridine is known as an aromatic component of coffee. The content of pyridine in the Indonesian coffee (A) is twice or more higher than those from elsewhere, indicating that it is a characteristic component in Indonesian coffee.

High end GC-MS system (GC-TOFMS): JMS-T200GC

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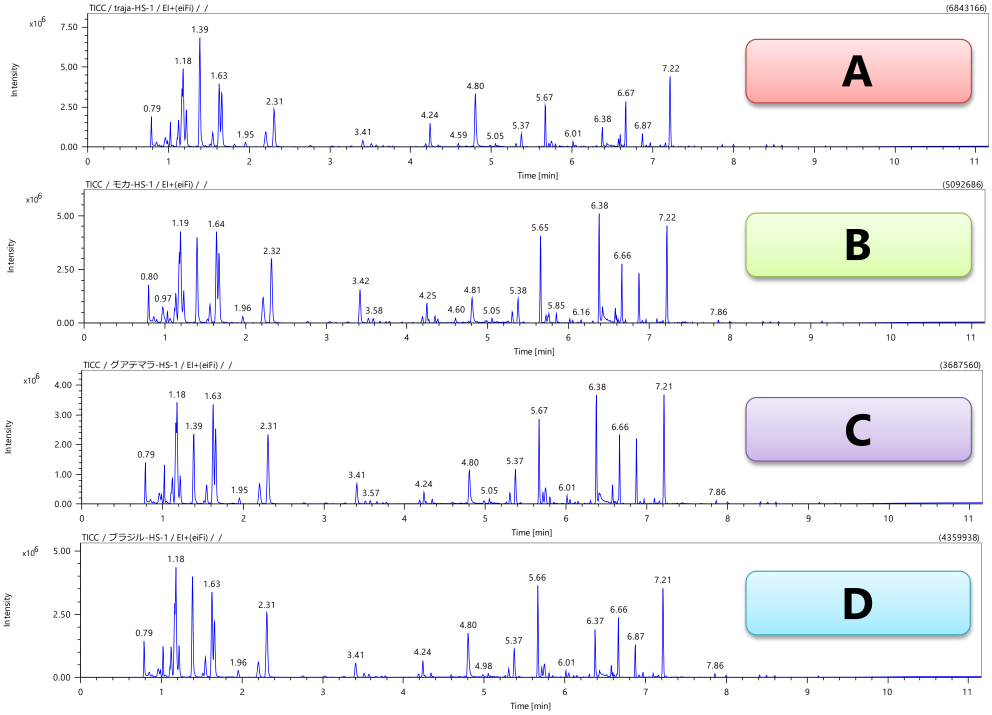

Figure 1. TIC chromatograms.

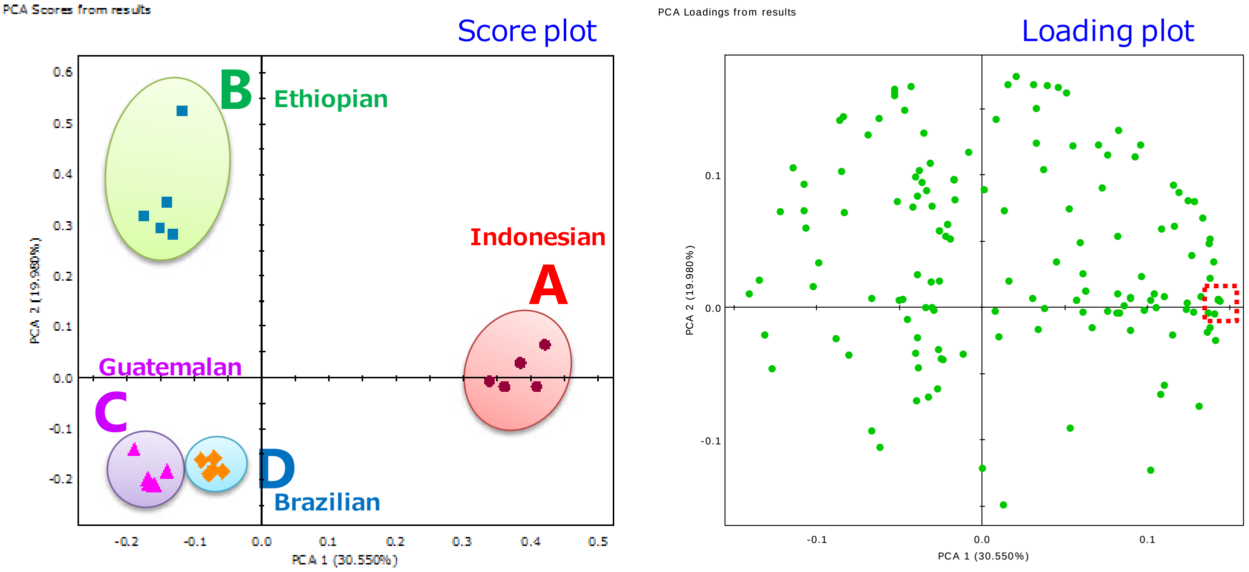

Figure 2. PCA score plot and loading plot.

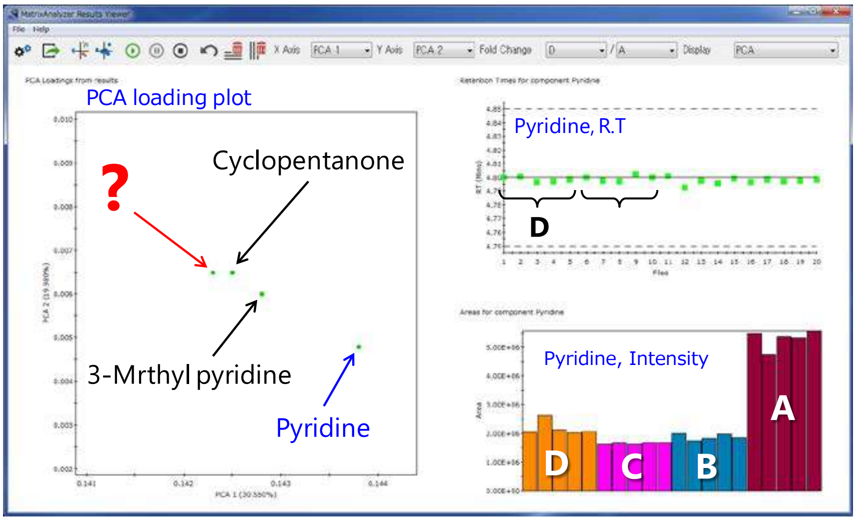
MatrixAnalyzer Results Viewer
File Help
X Axis
PCA 1
Y Axis
PCA 2
Fold Change
D
A
Display
PCA
PCA Loadings from results
PCA loading plot
?
Cyclopentanone
3-Mrthyl pyridine
Pyridine
PCA 2 (19.980%)
PCA 1 (30.550%)
0.141
0.142
0.143
0.144
Retention Times for component Pyridine
Pyridine, R.T
RT (Mins)
D
Files
Areas for component Pyridine
Pyridine, Intensity
Area
D
C
B
A

Figure 3. Enlarged view of the PCA loading plot

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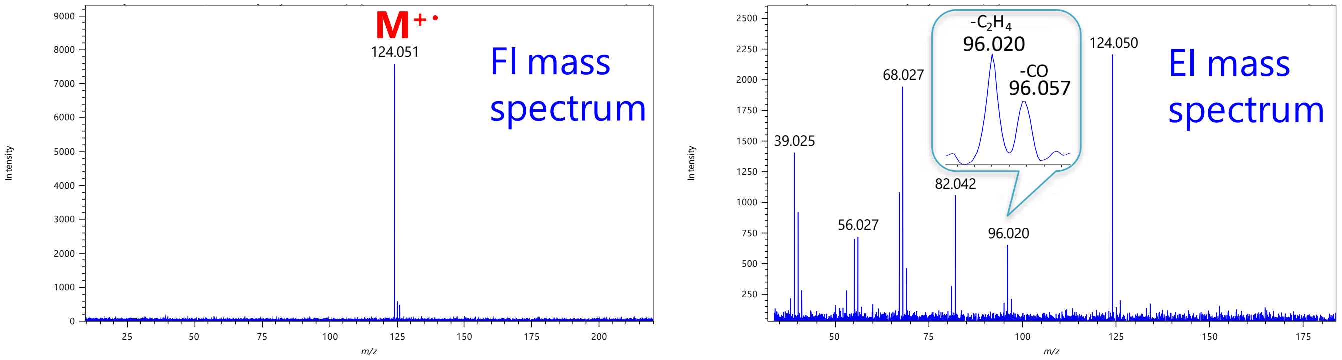
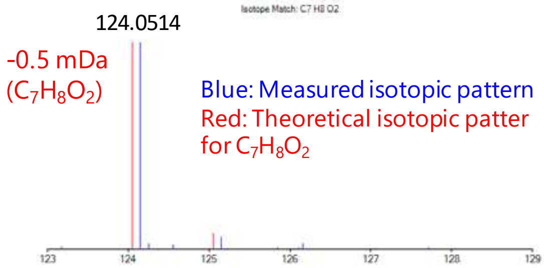


FI Accurate mass measurement result



EI Accurate mass measurement result

Mass	Formula	Calculated Mass	Mass Error [mDa]	DBE
39.0246	C3 H3	39.0229	1.7	2.5
56.0270	C3 H4 O	56.0257	1.3	2.0
68.0268	C4 H4 O	68.0257	1.1	3.0
82.0416	C5 H6 O	82.0413	0.3	3.0
96.0204	C5 H4 O2	96.0206	-0.2	4.0
96.0571	C6 H8 O	96.0570	0.1	3.0

Figure 4. EI and FI mass spectra and exact mass measurement results for the unknown component in the Indonesian coffee.

When a NIST library search was used for one of the 4 components shown in Figure 3 (marked by ? In the figure), the Match Factor was low at 682 for the top candidate, suggesting that this component is not registered in the NIST library database. Thus, the molecular formula of this component was estimated by soft ionization (FI). The structural formula was also estimated by calculating the composition of fragment ions observed by EI.

Figure 4 shows the FI mass spectrum, its isotopic pattern and exact mass analysis results as well as the EI mass spectrum and its exact mass analysis results. The peak at *m/z* 124 observed in the FI mass spectrum was subjected to exact mass and isotopic pattern analysis and was estimated to have a formula of $C_7H_8O_2$ and an unsaturation level of 4. Because the level of unsaturation was an integer, it was determined that the peak at *m/z* 124 was the molecular ion. When the EI fragment ions were subjected to exact mass analysis using each element and its quantity of $C_7H_8O_2$, the compositions of all fragment ions were determined. This also suggests that the formula for this unknown component is $C_7H_8O_2$. The structure shown in Figure 5 was estimated from the formulas of the EI fragment ions. The peak at *m/z* 96 observed as an EI fragment ion was a doublet peak produced by desorption of CO and ethylene from the molecular peak. High resolution MS can identify fragment ions having the same integer value (CO and ethylene are both 28 u) by determining their exact masses. The results demonstrate that accurate composition determination for the EI fragment ions makes it possible to estimate the structure of an unknown component.

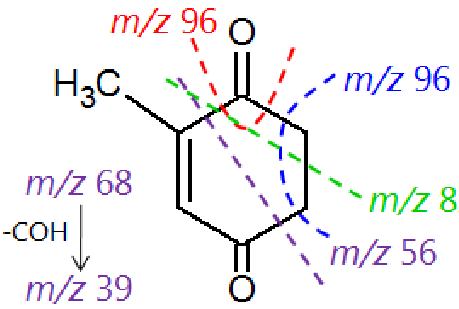


Figure 5. Estimated structural formula f
or the unknown component in Indonesian coffee

[Summary]

Multiple classification PCA can extract characteristic components that distinguish multiple samples. If any extracted component is not registered in the NIST library database, low resolution GC-MS is unable to identify the component. For these situations, it is effective to use a high resolution GC-MS capable of exact mass analysis combined with soft ionization to estimate the molecular formula and with EI to estimate the structural formula.

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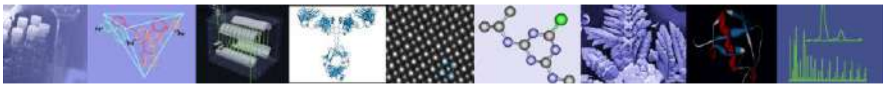


AccuTOF-GCv Series

SPME-GCxGC/HRTOFMS Analysis of Tequila

Introduction

The JEOL "AccuTOF GCv 4G" is a third generation GC/HRTOFMS system with high speed data acquisition capabilities of up to 50Hz which makes it well suited as the detector for comprehensive 2-dimensional GC (GCxGC) measurements. Along with the high speed data acquisition, this MS system also provides high mass resolution, accurate mass measurements, and high sensitivity, all simultaneously. Consequently, this GCxGC/HRTOFMS system is a powerful tool for the qualitative analysis of complicated samples.

In this work, we measured commercially available tequila samples using GCxGC/HRTOFMS combined with solid-phase micro-extraction (SPME) preparation.

Experimental

Sample information and measurement conditions are shown in Figure 2 and Table 1.

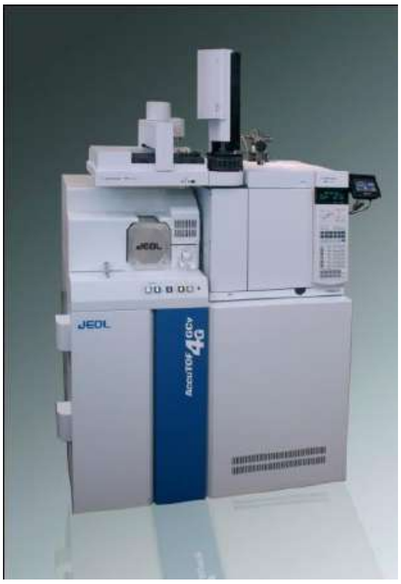


Fig. 1. GCxGC/HRTOFMS system.

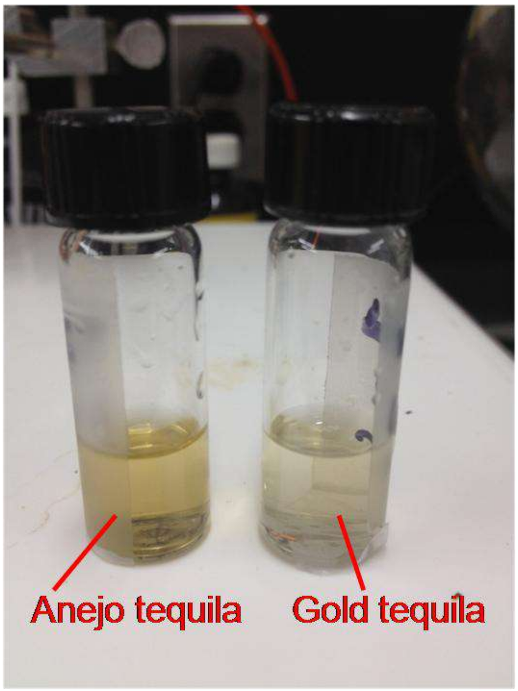


Fig. 2.   Tequila samples.

Condition	SPME-GCxGC-EI
Sample	1. Anejo tequila 2. Gold tequila
SPME	50/30 um DVB/CAR/PDMS (SUPELCO)
SPME condition	10 min at room temparature
GCxGC system	ZX2 thermal modulator (ZOEX)
1st column	Rxi-5SilMS, 30 m x 0.25 mm, 0.25 um
2nd column	Rxi-17SilMS, 2 m x 0.15 mm, 0.15 um
Modulator loop	Deactivated fused silica, 1.5 m x 0.15 mm
Modulator period	10 sec
Modulator duration	400 msec
Inlet pressure	200 kPa
Inlet mode	Split 10:1
Oven temp.	50 C (1 min) -> 3 C/min -> 250 C
GC-TOFMS system	AccuTOF GCv 4G (JEOL)
Ionization mode	EI+
Ionization condition	Ionization voltage: 70 V Ionization current: 300 uA
Ion source temp.	250 C
GC-ITF temp.	280 C
m/z range	*m/z* 35-500
Acquisition time	20 msec (50 Hz)
Sampling time	0.25 nsec (4 GHz)
External calibrant	*m/z* 207.0329 (column background)

Table 1. Measurement condition.

We analyzed two tequila samples (Figure 2), anejo and gold tequila. The anejo tequila is an aged tequila that is more expensive than the typical gold tequila. The SPME sample preparation step consisted of immersing the SPME fiber in the pure tequila for 10 minutes at room temperature. Afterwards, the SPME sample was measured using GCxGC/EI method (Table 1).

Results

The 2-dimensional total ion chromatograms (2D TICs) for each tequila sample are shown in Figures 3 and 4, respectively. Both TICs showed the presence of a wide variety of components in the sample.

The red circles in each 2D TIC, referred to as “Blobs,” show the detected chemical components and include

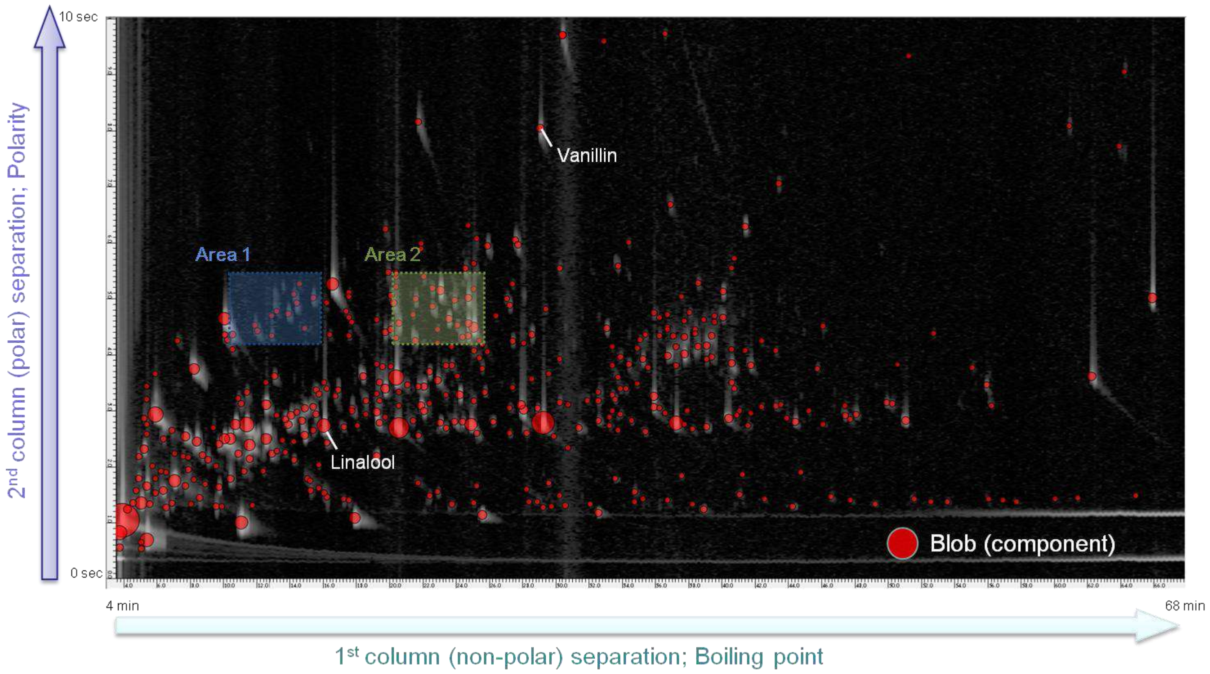


Fig. 3. GCxGC/EI TIC chromatogram of an anejo tequila.

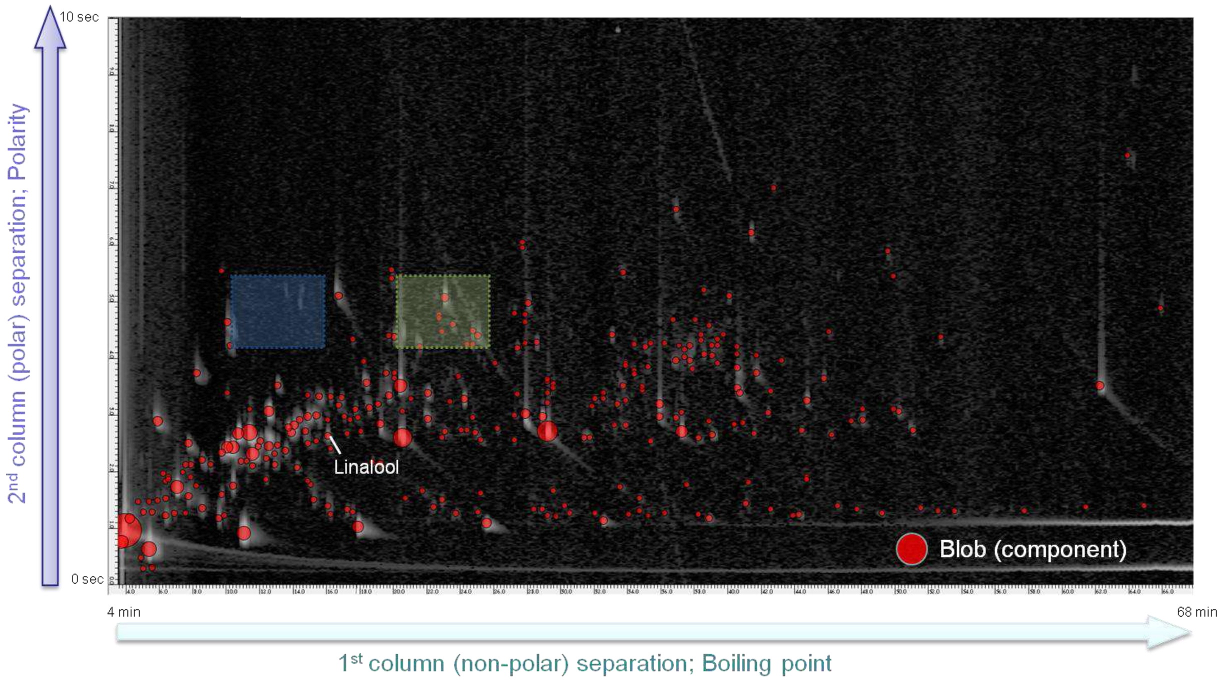


Fig. 4. GCxGC/EI TIC chromatogram of a gold tequila.

the EI mass spectrum for each component. The “Blob” size reflects the sum of the ion peak intensities for each chemical component.

As expected, the anejo tequila showed a more complex 2D TIC (i.e. more chemical components) than in the gold tequila 2D TIC image, which was the result of the longer anejo aging process. As an example of this, Area 1 (Blue region) and Area 2 (Green region) are highlighted in Figures 3 and 4.

Next, a NIST library search for all of the detected blobs was carried out for each sample. The NIST library search results are shown in Table 2. The anejo tequila had 409 chemicals detected in the 2D TIC with 236 of them (57.7%) identified with match factors of over 700, which is typically sufficient for reliable chemical identification. As for the gold tequila, 291 chemicals were detected with 141 of them (48.5%) identified with match factors of over 700.

In most cases, it is sufficient to use the NIST search results for identifying the compounds in the GCxGC TIC image. This step is easy to do as all blobs over the whole TIC image or for specific regions can be selected at once, and then a NIST library search can be carried out to find the best spectral matches. Afterwards, the GC Image software can then be used to automatically label each blob with the best match from the database search. Figure 5 shows the chemical identifications for a number of the blobs in the narrow Areas 1 and 2 TIC regions based on the NIST library search result.

	Component number	
NIST Library Match Factor	**Anejo tequila**	**Gold tequila**
Over 900	**31**	**23**
900-800	**101**	**57**
800-700	**104**	**61**
700-600	85	60
600-500	63	66
500-400	18	21
400-300	7	3
SUM	409	291

Table 2. NIST search library result.

Figure 6 shows a measured EI mass spectrum that was very similar to the NIST data for linalool, which showed a match factor of 922. Linalool is a naturally occurring monoterpene alcohol that is found in many plants. This compound is widely used as a flavoring agent for many kinds of foods. The molecular ion for linalool was not observed in the EI mass spectrum. However, the fragment ion resulting from the dehydration of the molecular ion ($[M\text{-}H_2O]^+$) was observed and showed a mass accuracy of 1.57 mDa compared to the calculated value for $C_{10}H_{16}$ while using an external one-point drift compensation for the mass calibration. Figure 5 shows a measured EI mass spectrum that was very similar to the NIST data for vanillan, which showed a match factor of 905.

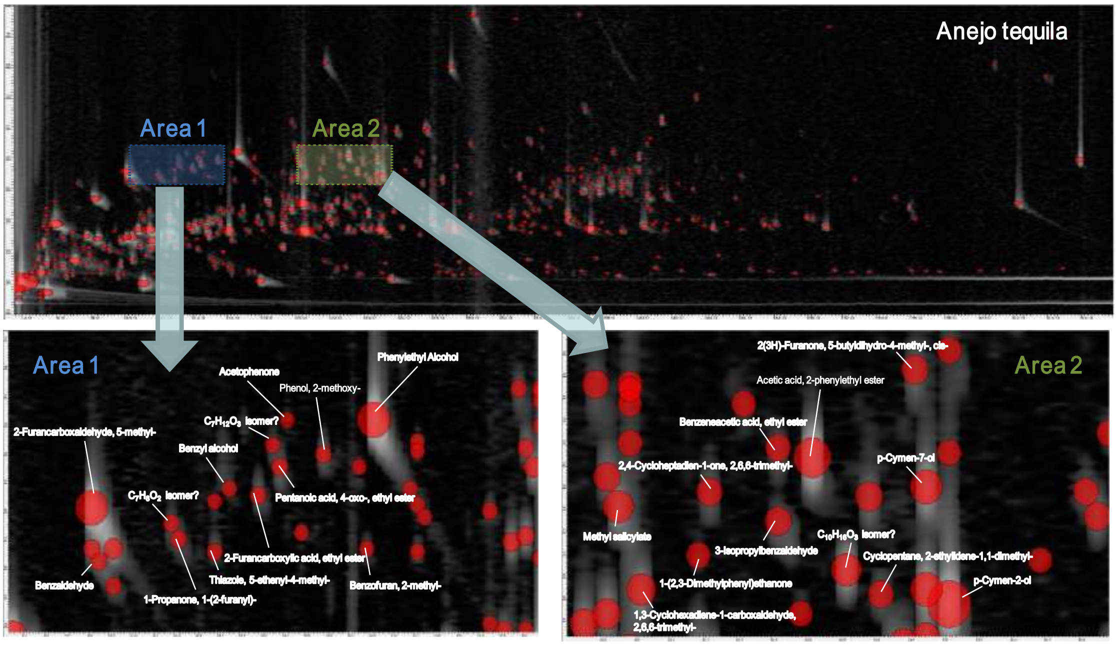
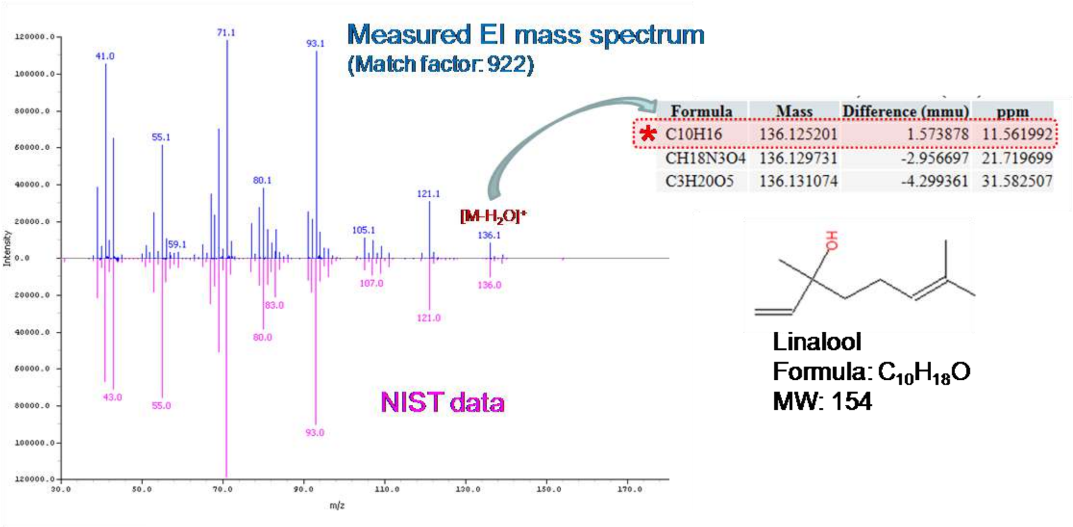


Fig. 5. Example of chemical identifications result.

Additionally, the molecular ion showed a mass accuracy of 0.88 mDa for the elemental composition of $C_8H_8O_3$. Vanillin is a phenolic aldehyde that was only detected in the anejo tequila (Figure 3). These results clearly show that even for 50Hz GCxGC measurement data, we can obtain normal EI mass spectral patterns that are directly comparable to NIST database mass spectra and high mass accuracy information to help further confirm the identity of unknown compounds through elemental composition calculations.

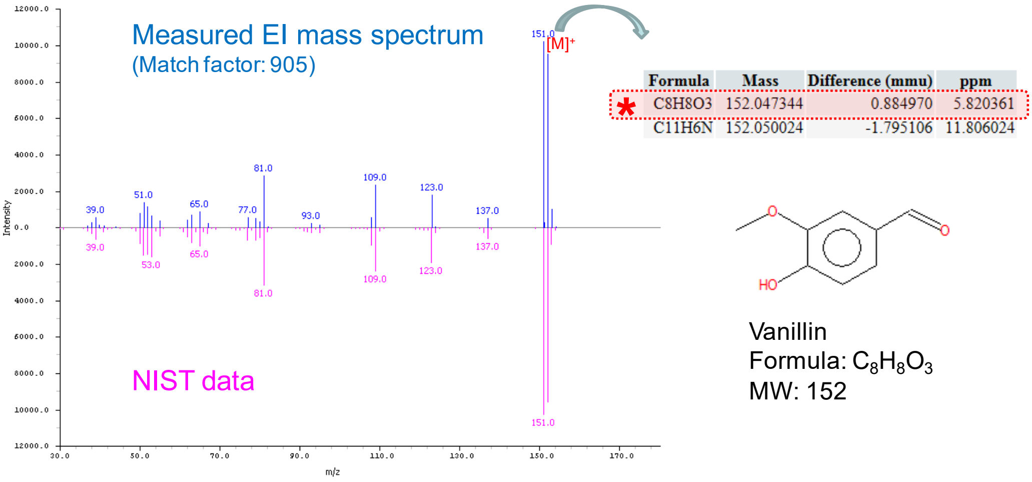
Conclusion

The AccuTOF GCv 4G allows 50Hz GCxGC measurements with high sensitivity, high mass resolution and high mass accuracy, all simultaneously. Additionally, we can do NIST library searches using GCxGC data in exactly the same way as for regular 1D GC/MS. The AccuTOF GCv 4G coupled with the 2D GC technique is an extremely useful tool for the qualitative analysis of complicated samples.



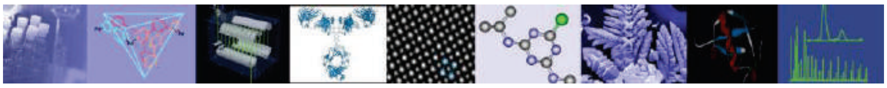
	Formula	Mass	Difference (mmu)	ppm
*	C10H16	136.125201	1.573878	11.561992
	CH18N3O4	136.129731	-2.956697	21.719699
	C3H20O5	136.131074	-4.299361	31.582507

Fig. 6. Measured EI mass spectrum and NIST data of Linalool.



	Formula	Mass	Difference (mmu)	ppm
*	C8H8O3	152.047344	0.884970	5.820361
	C11H6N	152.050024	-1.795106	11.806024

Fig. 7. EI mass spectrum and NIST data of Vanillin.



AccuTOF-GCv Series

Aroma Oil Analysis using GCxGC-HRTOFMS Performance Test for AccuTOF GCv 4G

Introduction

The AccuTOF GCv 4G is JEOL's third generation high resolution GC-TOFMS. New, enhanced features of the system include:

1) Recording speed: up to 50 spectra/sec
2) Mass resolution: 8,000 or more (*m/z* 614, FWHM)
3) Mass accuracy: 1.5 mmu or 4 ppm
4) Mass range: *m/z* 4 to 5,000

Comprehensive 2D GC (GCxGC) is a chromatographic separations technique that uses 2 columns with different polarities arranged in a series. Featuring higher resolution than conventional capillary GC analysis, it is a powerful tool for the measurement of multiple components in a complex mixture. However, because there is a cryo-trap before the 2nd column, the resulting peaks in the chromatograms are extremely narrow. As a result, the system requires a detector capable of high speed data recording. The TOFMS is an ideal detector for the 2D GC system.

In this work, we analyzed aroma oil using a GCxGC-HRTOFMS system, in which the AccuTOF GCv 4G was used with a Zoex GCxGC system to examine the spectrum recording speed and mass accuracy.

Instrument	JMS-T100GCV 4G (JEOL Ltd.)
	KT2004 (Zoex Corporation)
Injection mode	Split 200 : 1
Injection temp.	270 °C
Oven temp.	50 °C(3min) → 5 °C/min → 270 °C(8min hold)
Injection volume	0.2 µL
Column set	1st: BPX-5 (30 m x 0.25 mm, 0.25 µm)
	2nd: BPX-50 (2 m x 0.1 mm, 0.1 µm)
Modulation period	6 sec
Ionization mode	EI+: 70 eV, 300 µA
Ion source temp.	270 °C
m/z range	*m/z* 35 - 500
Data acquisition	0.02 sec (50 Hz)

Table 1. Measurement condition

Experimental

For the sample, a commercial product of tea tree oil, a type of aroma oil, was used without treatment. Table 1 shows the measurement conditions used for the analysis. GC Image (Zoex) was used for processing of the GCxGC data.

Results and Discussion

Figure 1 shows a 2D map created from the aroma oil TIC. The compounds identified from each EI mass spectrum

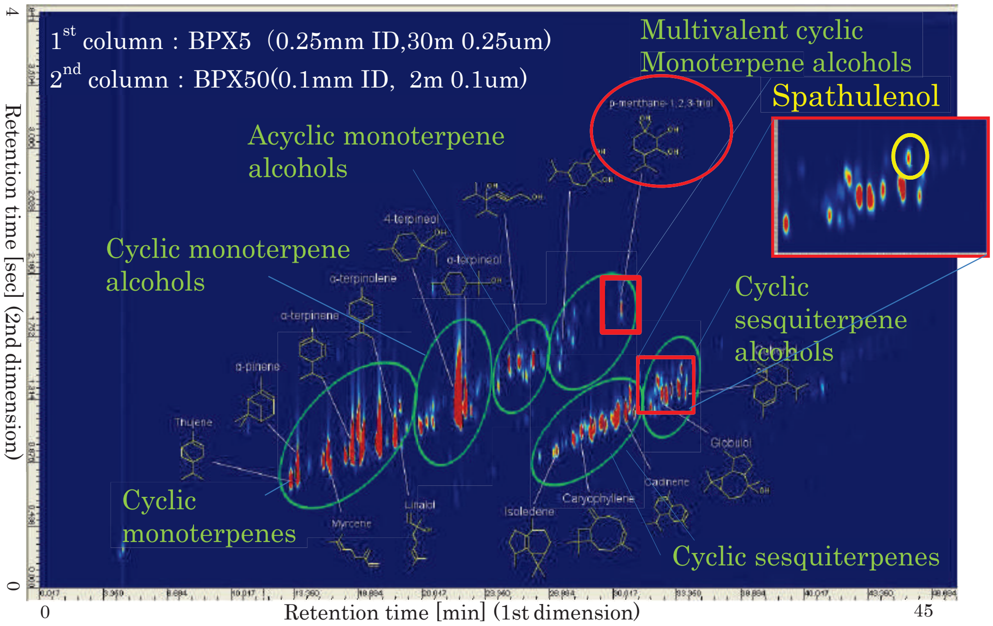


Figure 1. 2D map of tea tree oil sample analyzed by GCxGC-HRTOFMS

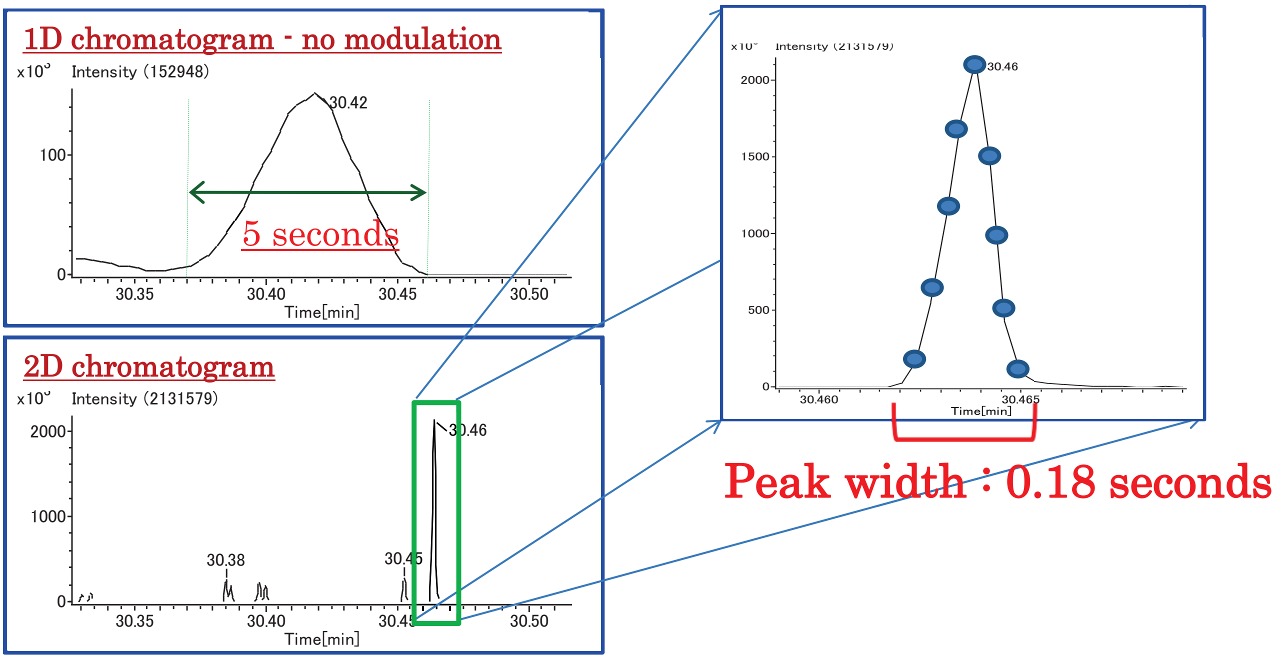


Figure 2. Magnified peak of p-menthane-1,2,3-triol

and their corresponding structures are marked on the 2D map.

Because the column set used for the analysis had a nonpolar 1st column and a polar 2nd column, the components were eluted with reference to their boiling point in the horizontal axis and their polarity in the vertical axis. Figure 1 shows cyclic and acyclic monoterpenes and monoterpene alcohols in groups from 12 to 30 minutes in the horizontal axis. The cyclic sesquiterpenes and cyclic sesquiterpene alcohols were observed between 25 and 35 minutes in the 1st dimension.

Recording speed

The recording speed was examined using the peak width in the chromatogram of p-menthane-1,2,3-triol that was eluted at approximately 30 minutes in Figure 1. Figure 2 shows the 1D and 2D chromatograms of p-menthane-1,2,3-triol at the elution time with and without modulation on the same column set. The figure also shows a magnified chromatogram of the p-menthane-1,2,3-triol peak in the 2D chromatogram.

In the 1D chromatogram without modulation, the peak with p-menthane-1,2,3-triol as its major component had a peak width of approximately 5 sec. Meanwhile, in the 2D chromatogram, the p-menthane-1,2,3–triol had an extremely narrow peak width of approximately 0.18 sec. This demonstrates that at the recording speed at 50 Hz, the system was able to acquire sufficient data points on the chromatogram, 9 points as shown in the magnified view in Figure 2, compared to the recording speed at 2.5 to 10 Hz that is typically used in 1D GC-MS data acquisition.

Exact mass accuracy

Next, the mass accuracy was examined using the mass spectrum of spathulenol ($C_{15}H_{24}O$, exact mass: 220.18271), a cyclic sesquiterpene that eluted at approximately 33 min in Figure 1. Figure 3 shows the measured mass spectrum for this compound. Table 2 shows the error (mDa) between the measured exact mass and the expected exact mass for the molecular ion when the same sample was analyzed 5 different times. The error ranged from 0.46 to 0.78 mDa, showing extremely high levels of accuracy and stability. These results demonstrate that the system is capable of estimating the composition with high precision during the high speed data acquisition that is required for GCxGC analyses.

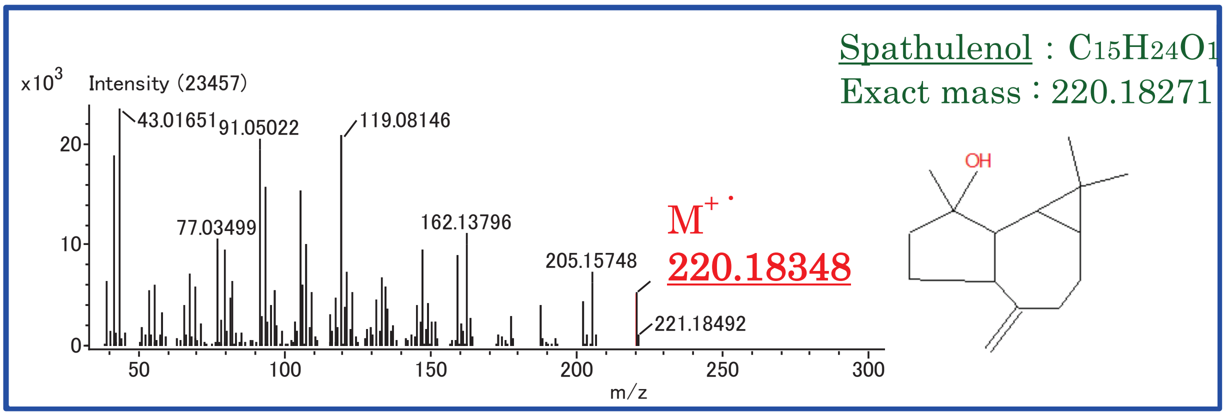


Figure 3. Mass spectrum of spathulenol

No.	Accurate mass (Da)	Error (mDa)
1	220.18348	0.77
2	220.18337	0.65
3	220.18326	0.55
4	220.18349	0.78
5	220.18318	0.46

Table 2. Errors between exact mass and accurate mass of spathulenol

JEOL MS Data Sheet

MS Tips

Mass Spectrometry Application Group
Mass Spectrometry Business Unit
JEOL Ltd. www.jeol.com

No.167 (U , 06/'10)

JMS-T100GCV Application Data

Fast GC/TOFMS Analysis of Organophosphorus Pesticides

Introduction

With this MSTips, we examined the Fast GC analysis of organophosphorus pesticides by using gas chromatograph – high resolution time-of-flight mass spectrometer (GC-HRTOFMS) JMS-T100GCV which enables high speed data acquisition. Our findings on the analysis of pesticide residues in food using GC-HRTOFMS based on this examination are reported.

Sample and analysis conditions

Extract of frozen pumpkin was used to simulate food matrix. Water (5 g) and acetonitrile (10 mL) was added to 10 g of frozen pumpkin and homogenized. The extraction procedure according to “QuEChERS” method was then performed. The extract (in acetonitrile) was purified according to the “Simultaneous Test Methods for Agrochemicals by GC/MS (Agricultural Products)”. The resulted residue was dissolved in 2 mL of acetone/hexane = 1/1. Finally, the standard pesticide mixture was added to the concentration equivalent to 0.01 ppm (per each analyte) in the original frozen pumpkin.

Table 1 GC-HRTOFMS analysis conditions.

Instrument	JMS−T100GCV (JEOL Ltd.)
Quantitative software	Escrime (JEOL Ltd.)
Injection mode	Pulsed Splitless (250 kPa, 1.1 min)
Injection temp.	250°C
Oven temp. program	50°C(0.5min)→45°C/min→125°C →20°C/min→300°C(5min)
Injection volume	1µL
GC column	ZB−5ms, 20m×0.18mm、0.18µm
Carrier gas	He, 0.7mL/min (Constant flow mode)
Ionization mode	EI+ (70eV, 300µA)
m/z range	*m/z* 40−450
Data acquisition speed	0.1 sec (10 Hz)

The analysis conditions are shown in Table 1. The Fast GC condition was optimized by referring to the “GC Condition (example 2)” in the Office Memo of the Ministry of Health, Labour, and Welfare ”Test Method for Organophosphorus Pesticide Residues in Foods” dated March 7, 2008(1).

Results and Discussion

Under the Fast GC condition shown above, the retention times of methamidophos, the first eluting analyte, and coumaphos, the last eluting analyte, were 3.64 min and 10.87 min respectively. The total analysis time was shortened to 16 min; approximately half of 31.5 min under conventional GC condition.

The reproducibility of 5 repetitive analyses of the sample is summarized on Table 2. The mass chromatogram for quantitation was generated with the m/z window of (calculated exact m/z) ± 0.025 for each analyte. The C.V. % of the mass chromatogram peak areas for 5 analyses for each analyte was 7.7 % or less, showing that the limit of quantitation (LOQ) is well below 0.01 ppm in frozen pumpkin for all analytes. The average error of measured accurate *m/z* of the quantitation ion is below 2 mDa for most analytes, showing that the confirmation of all analytes based on measured accurate *m/z* is possible.

Reference

1） Test Method for Organophosphorus Pesticide Residues in Foods, M.H.L.W, March 7, 2008.
2） Ubukata, M., et al., Abstract of The 32nd Workshop for Pesticide Residue Analysis, P217-225 (2009).

Table 2 Results of quantitative analysis with Fast GC technique.

No.	Pesticides	Retention time (min)	Qualitative ion (*m/z*)	Average mass error (n=5) (mDa)	Mass chromatogram peak area C.V. (n=5) (%)
1	Methamidophos	3.64	94.0058	1.3	3.2
2	Dichlorvos	3.73	109.0055	0.9	2.2
3	Acephate	4.77	136.0164	1.1	3.5
4	Omethoate	5.68	156.0010	0.8	3.1
5	Ethoprophos	5.93	157.9625	1.1	6.1
6	Cadusafos	6.21	158.9703	0.3	2.1
7	Monocrotophos	6.14	127.0160	1.2	3.4
8	Salithion	6.14	216.0010	0.6	4.7
9	Phorate	6.26	75.0269	1.4	3.2
10	Thiometon	6.39	88.0347	1.3	4.5
11	Dimethoate	6.44	87.0158	1.0	3.8
12	Terbufos	6.70	230.9737	1.1	6.5
13	Diazinon	6.74	137.0715	0.4	4.4
14	Cyanophos	6.69	243.0119	0.5	3.1
15	Ethylthiometon	6.87	88.0347	0.8	5.5
16	Etrimfos	6.91	292.0647	0.8	2.6
17	Iprobenfos	7.03	91.0566	0.6	3.6
18	Formothion	7.08	124.9826	0.9	4.5
19	Dichlofenthion	7.18	279.0012	1.4	3.4
20	Chlorpyrifos-methyl	7.24	285.9261	0.8	2.2
21	Tolclophos-methyl	7.32	264.9855	0.8	3.7
22	Pirimiphos-methyl	7.52	290.0728	1.1	5.3
23	Fenitrothion	7.56	124.9826	1.4	4.3
24	Dimethylvinphos-E	7.59	294.9694	1.1	1.8
25	Malathion	7.63	124.9826	1.0	4.2
26	Chlorpyrifos	7.71	196.9202	0.9	1.3
27	Dimethylvinphos-Z	7.73	294.9694	0.9	0.6
28	Fenthion	7.76	278.0200	0.9	2.1
29	Fosthiazate-1	7.95	195.0119	1.4	6.3
30	Chlorfenvinphos-E	8.03	266.9381	0.9	1.9
31	Fosthiazate-2	7.98	195.0119	1.0	3.3
32	Isofenphos	8.12	213.0317	0.9	4.9
33	Chlorfenvinphos-Z	8.13	266.9381	1.3	1.0
34	Phenthoate	8.20	273.9887	1.1	1.0
35	Quinalphos	8.21	146.0480	1.6	2.3
36	Bromophos (Bromophos-methyl)	8.34	358.9070	0.9	3.4
37	Propaphos	8.35	219.9959	1.3	0.8
38	Methidathion	8.35	145.0072	1.3	3.1
39	Vamidothion	8.41	145.0561	1.3	5.1
40	Butamifos	8.51	286.1031	2.3	5.9
41	Phenamiphos	8.53	303.1058	1.1	1.3
42	Prothiofos	8.62	266.9470	1.8	4.8
43	Profenofos	8.66	207.9112	0.5	2.0
44	Isoxathion	8.88	105.0340	0.7	7.7
45	Fensulfothion	9.03	292.0351	1.1	2.6
46	Ethion	9.10	230.9737	1.5	4.6
47	Sulprofos	9.26	322.0285	1.7	4.4
48	Cyanofenphos	9.37	156.9877	0.4	4.0
49	Edifenphos	9.40	109.0123	0.5	2.1
50	Pyridaphenthion	9.82	340.0647	1.1	5.7
51	EPN	9.92	156.9877	0.4	2.2
52	Phosmet	9.91	160.0434	0.7	3.2
53	Phosalone	10.24	182.0009	0.8	4.1
54	Azinphos-methyl	10.29	132.0449	1.0	2.9
55	Azinphos-ethyl	10.58	132.0449	1.2	7.2
56	Pyraclofos	10.64	360.0464	2.8	4.5
57	Coumaphos	10.87	362.0145	2.3	5.4

JEOL MS Data Sheet

MS Tips

No.147

Mass Spectrometry Application Group
Mass Spectrometry Business Unit
JEOL Ltd.

JMS-T100GCV Application Data

Qualitative analysis of pyrazole pesticides in tea leaf by using FastGC-HRTOFMS

[Introduction]

FastGC method is a very useful technique for rapid GC analysis. On the other hand, GC-TOFMS has the capability of very fast data acquisition in comparison with other types of mass spectrometers. Therefore, TOFMS is most suitable to combine with the FastGC technique. In combination with the high resolution capability (HR-TOFMS) we can obtain very accurate spectra with exact m/z determination.

In this application note, we describe the qualitative and quantitative analysis by FastGC/HRTOFMS of pyrazole pesticides (Fipronil, Ethiprole, Pyraflufen ethyl and Tebfenpyrad) in tea leaf. We confirm that rapid analysis with high sensitivity is easy to perform and very useful for fast screening.

[Sample and method]

Measurement conditions are shown in Table 1. Tea leaf (5g) was prepared using the multiresidue method for agricultural chemicals by GC/MS published by Ministry of Health, Labour and Welfare, Japan. Pyrozole pesticides were added to make 0.01, 0.05 and 0.1ppm solution in the prepared solution from tea leaf. These concentrations in solution are equivalent to 4, 20 and 40ppb in tea leaf. Each sample was analyzed 3 times to check the reproducibility.

Table 1 GC/MS measurement conditions.

Instrument	JMS-T100GCV (JEOL)
Quantitative software	Escrime (JEOL)
Injection mode	Splitless
Injection temp.	250℃
Oven temp. program	40℃(1min) → 50℃/min → 300℃(3.8min)
Injection volume	1μL
Column	DB-5, 10m x 0.18mm, 0.18μm
Carrier gas	He, 0.7mL/min, Const. flow
Ionization mode	EI+, 70eV, 300μA
Ion source temp.	250℃
m/z range	m/z 35 - 500
Spectrum recording time	0.1sec

[Results and discussion]

Fig.1 shows TIC chromatogram and mass chromatograms of each pesticide. Pyrazole pesticides are detected within 6 minutes by using the FastGC method. Expanded mass chromatogram of Fipronil is shown in the right side of Fig.1. The peak width becomes very narrow in the FastGC methods.

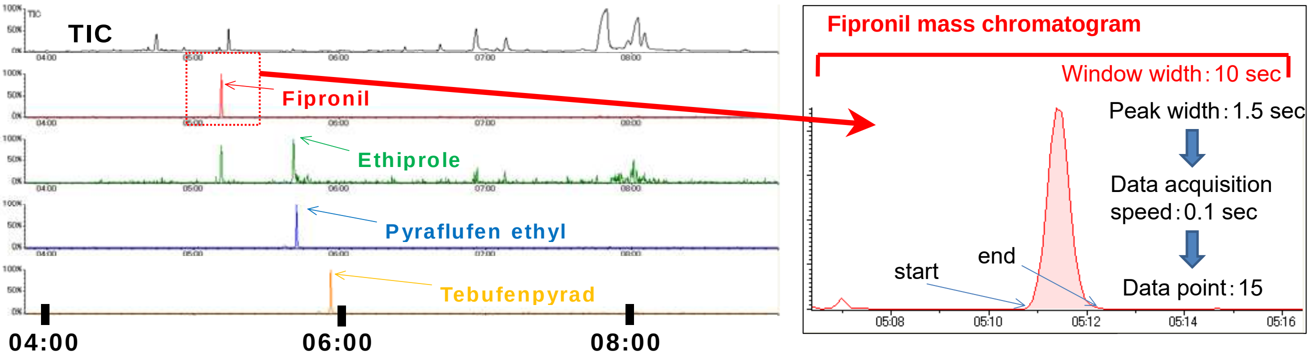


Fig.1 TIC chromatograms and Mass chromatograms

Now, the maximum recording interval on JMS-T100GCV is 0.04 seconds/spectrum (25Hz). When 0.1 seconds/spectrum (10Hz) of recording interval is used in this analysis, about 15 data points are acquired per chromatographic peak and this is enough to get good peak profile.

Mass spectrum of Fipronil is shown in Fig.2. Chemical backgrounds from tea leafs are observed prominently even at very low concentrated solution. However, characteristic ions of Fipronil such as *m/z* 350.95, 366.94 and 419.94 are observe and Fipronil is identified as first choice using NIST database search even in 0.01 ppm sample solution (4 ppb in tea leaf). In addition, mass accuracy for *m/z* 350.95, 366.94 and 419.94 is within 2.0x10^{-3}u. Table 2 shows the mass accuracy for characteristic ions of each pyrazole pesticide at different concentrations.

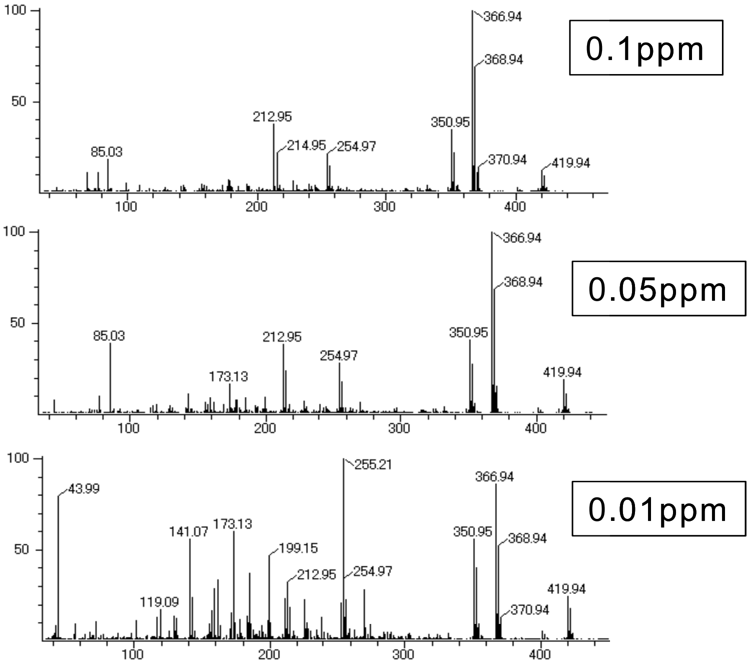


Fig.2 Mass spectra of Fipronil.

JMS-T100GCV can easily obtain good data with high spectrum sensitivity and high mass accuracy even if sample includes chemical contaminants. Identification using accurate mass is very useful in addition to the database search.

Table 2 Results of exact mass measurements.

Fipronil

ion	$C_{11}H_4Cl_2F_3N_4S$	$C_{11}H_4Cl_2F_3N_4OS$	$C_{12}H_4Cl_2F_6N_4S$
Calc. exact mass	350.9486	366.9435	419.9438

ppm	Meas. exact mass	Error (10^{-3}u)	Meas. exact mass	Error (10^{-3}u)	Meas. exact mass	Error (10^{-3}u)
0.1	350.9473	−1.3	366.9417	−1.8	419.9435	−0.3
0.05	350.9472	−1.4	366.9423	−1.2	419.9425	−1.3
0.01	350.9474	−1.2	366.9431	−0.4	419.9449	1.1

Pyraflufen ethyl

ion	$C_{12}H_8Cl_2F_3N_2O_2$	$C_{13}H_9ClF_3N_2O_4$	$C_{15}H_{13}Cl_2F_3N_2O_4$
Calc. exact mass	338.9915	349.0203	412.0205

ppm	Meas. exact mass	Error (10^{-3}u)	Meas. exact mass	Error (10^{-3}u)	Meas. exact mass	Error (10^{-3}u)
0.1	338.9917	0.2	349.0194	−0.9	412.0212	0.7
0.05	338.9911	−0.4	349.0184	−1.9	412.0207	0.2
0.01	338.9914	−0.1	349.0191	−1.2	412.0201	−0.4

Ethiprole

ion	$C_8H_4Cl_2F_3N_2$	$C_{11}H_5Cl_2F_3N_4S$	$C_{13}H_9Cl_2F_3N_4S$
Calc. exact mass	254.9704	351.9564	379.9877

ppm	Meas. exact mass	Error (10^{-3}u)	Meas. exact mass	Error (10^{-3}u)	Meas. exact mass	Error (10^{-3}u)
0.1	254.9722	1.8	351.9577	1.3	379.9894	1.7
0.05	254.9721	1.7	351.9547	−1.8	379.9885	0.8
0.01	254.9767	6.4	351.9563	−0.1	379.9897	2.0

Tebufenpyrad

ion	$C_7H_8ClN_2O$	$C_{17}H_{21}ClN_3O$	$C_{18}H_{24}ClN_3O$
Calc. exact mass	171.0325	318.1373	333.1608

ppm	Meas. exact mass	Error (10^{-3}u)	Meas. exact mass	Error (10^{-3}u)	Meas. exact mass	Error (10^{-3}u)
0.1	171.0343	1.8	318.1379	0.6	333.1617	0.9
0.05	171.0335	1.0	318.1383	1.0	333.1614	1.7
0.01	171.0333	0.8	318.1388	1.5	333.1616	0.8

[Reference]

M. Ubukata et al., Abstract of the 97th conference of the Japanese Society for Food Hygiene and Safety, page 20 (2009)

JEOL MS Data Sheet

MS Tips

Mass Spectrometry Application Group
Mass Spectrometry Business Unit
JEOL Ltd.

No.148

JMS-T100GCV Application Data

Quantitative analysis of pyrazole pesticides in tea leaf using FastGC-HRTOFMS

[Introduction]

FastGC method is a very useful technique for rapid GC analysis. On the other hand, GC-TOFMS has the capability to acquire data very fast in comparison with other types of mass spectrometer. Therefore, TOFMS is most suitable mass spectrometer to combine with FastGC method. In combination with the high resolution capability (HR-TOFMS) we can obtain very accurate spectra with exact m/z determination.

In this application note, we describe the qualitative and quantitative analysis by FastGC/HRTOFMS of pyrazole pesticides (Fipronil, Ethiprole, Pyraflufen ethyl and Tebfenpyrad) in tea leaf. We confirm that rapid analysis with high sensitivity is easy to perform and very useful for fast screening.

[Sample and method]

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Table 1 GC/MS measurement conditions.

Instrument	JMS-T100GCV (JEOL)
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Injection temp.	250℃
Oven temp. program	40℃(1min) → 50℃/min → 300℃(3.8min)
Injection volume	1μL
Column	DB-5, 10m x 0.18mm, 0.18μm
Carrier gas	He, 0.7mL/min, Const. flow
Ionization mode	EI+, 70eV, 300μA
Ion source temp.	250℃
m/z range	m/z 35 - 500
Spectrum recording time	0.1sec

[Results and discussion]

Fig.1 shows an expanded mass spectrum of a 0.01ppm sample solution (4ppb in tea leaf) of Fipronil. This spectrum shows the *m/z* 254.97 ion produced by Fipronil and the ion of m/z 255.21 produced by a contaminant. When low-resolution MS such as QMS is used, these ions can not be separated. However, as Fig.1 shows, HR-TOFMS can separate each ion easily.

Therefore, it is possible to create high-resolution mass chromatogram with narrow m/z window (±0.05 Da) in order to eliminate the influence of chemical background.

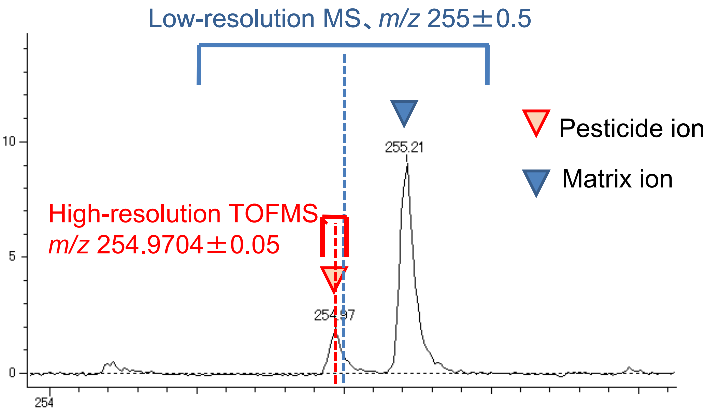

Fig.1 Mass spectrum of Fipronil.

Fig.2 shows high-resolution the mass chromatograms for each pesticide in a 0.01ppm sample solution.

Fig.3 shows the calibration curves and Table 2 shows the reproducibility (n=3) for each pesticide. Japanese default maximum regulated residues level (MRLs) for Fipronil is 2ppb and for Pyraflufen ethyl is 10ppb. The averaged S/N for each chromatographic peak in a 0.01 ppm sample solution (4ppb in tea leaf) is shown in Fig.2. For both Fipronil and Pyraflufen ethyl, this is almost 300. This S/N is enough to analyze them even if these concentrations are around the MRL value. Furthermore, the correlation coefficient for each pesticide is more than 0.997 and it shows very good linearity. The reproducibility (n=3) is shown in Table2. The variation coefficient C.V. (%), of about 10% for each pesticide at each concentration, demonstrates good reproducibility.

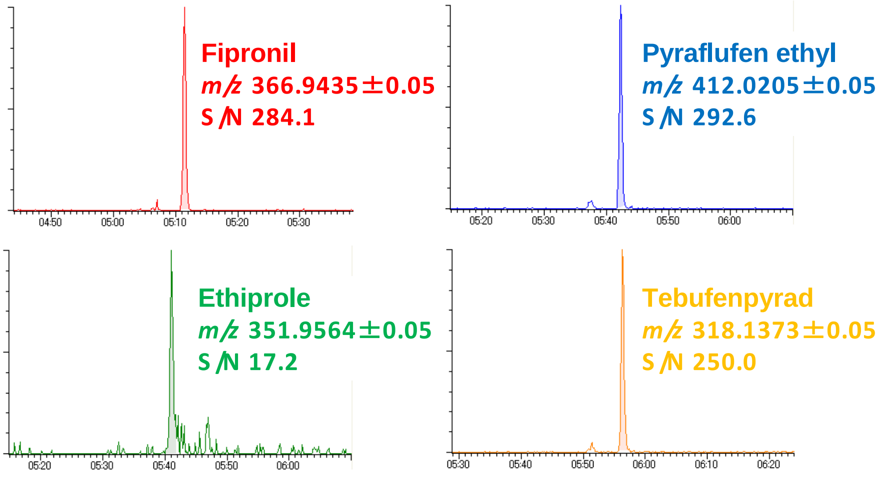

Fig.2 High-resolution Mass chromatograms of 0.01ppm

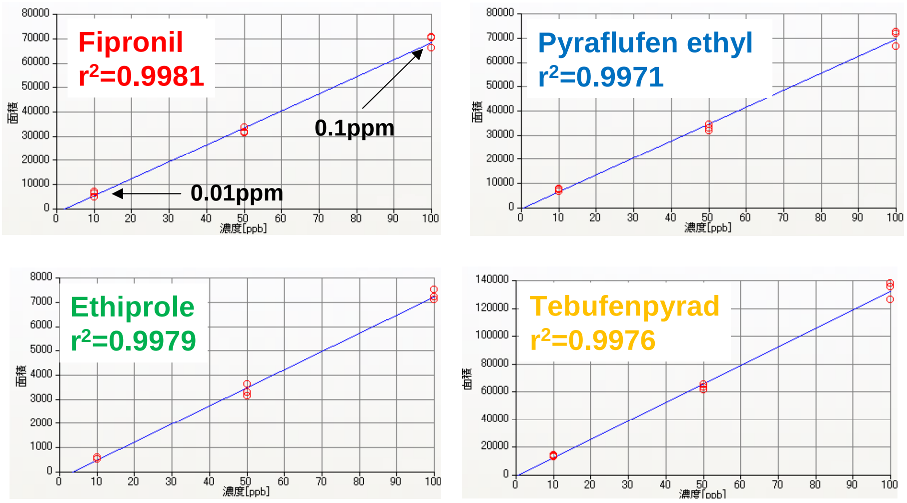

Fig.3 Calibration curves

Table 2 Results of quantitative analysis.

ppm	No.	Fipronil	Ethiprole	Pyraflufen ethyl	Tebufenpyrad
0.01	1	9.39	10.51	10.47	10.58
	2	11.37	10.51	11.49	11.26
	3	11.81	11.85	11.65	10.8
	Ave.	10.86	10.96	11.20	10.88
	C.V.(%)	11.87	7.06	5.71	3.19
0.05	1	49.73	47.36	49.57	49.84
	2	46.78	45.64	46.1	47.06
	3	47.37	51.84	49.57	48.35
	Ave.	47.96	48.28	48.41	48.42
	C.V.(%)	3.25	6.63	4.14	2.87
0.1	1	101.06	99.98	104.01	102.29
	2	95.44	98.32	95.63	95.43
	3	101.66	104	103.25	104.39
	Ave.	99.39	100.77	100.96	100.70
	C.V.(%)	3.45	2.90	4.59	4.65

This result shows that the JMS-T100GCV can easily obtain good quantitative result with high spectrum sensitivity, high mass accuracy and high resolution even if sample including chemical contaminants.

[Reference]

M. Ubukata et al., Abstract of the 97th conference of the Japanese Society for Food Hygiene and Safety, page 20 (2009)

JEOL MS Data Sheet

MS Tips

Mass Spectrometry Application Department,
Mass Spectrometry Business Unit
JEOL Ltd. www.jeol.com

No. 130

JMS-T100GC Application Data

Examining Selectivity using High Resolution Extracted Ion Current Chromatograms (EICC)

[Introduction]

Transformer oil containing no PCBs was diluted 10000 times, and then this solution was spiked with a mixture of commercially available PCBs (KC-500). The resulting sample was analyzed by using the JEOL AccuTOF-GC with FastGC/MS conditions. Afterwards, the data was examined by varying the mass range window (called “window width” in EICC generation) used for the PCB extracted ion current chromatograms (EICC) to determine if the effect of the background interference can be eliminated so that the analyte peaks are easily observed in the resulting data.

[Samples and Measuring Conditions]

Sample	KC-500 (0.1 ppm), transformer oil (diluted 10000 times)	
GC conditions	Sample inlet:	Splitless, 280℃
	Column:	DB-5, 10 m x 0.18 mm, 0.18 um
	He flow rate:	0.5 ml/min (fixed flow rate)
	Oven:	50℃(2 min) → 60℃/min → 280℃(2 min)
MS conditions:	MS:	JMS-T100GC AccuTOF GC
		Ionization mode: EI+ (ionization voltages: 70 eV, current: 300 uA)
	Mass range:	*m/z* 30 to 550
	Recording interval:	0.1 s (10 Hz)
	Temperature:	Ion source: 280℃, GC-ITF: 280℃

[Result and Discussion]

The window width for the EICC generation was varied to see if the chromatographic effect of the background interferences can be eliminated. Figure 1 shows the EICCs generated under low resolution (top) and high resolution (bottom) conditions. “Low resolution” and “high resolution” do not refer to the resolving power of the instrument, but instead refer to the different window widths used for the *m/z* 352.88 EICCs. The mass resolution levels calculated with the

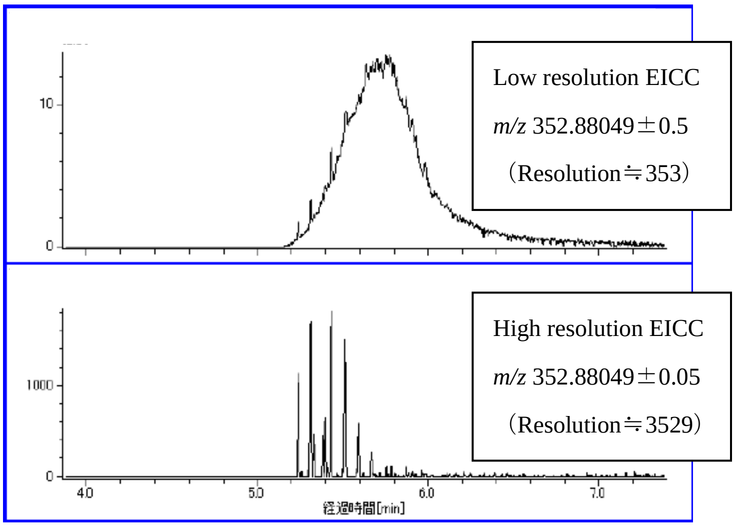

Figure 1. Comparison of mass chromatograms for penta-chlorinated PCBs in oil

different window widths are shown in Figure 1. The actual resolution for the data acquired by the AccuTOF GC during these sample measurements was R≧5000 @ *m/z* 293. As Figure 1 shows, the low resolution EICC (*m/z* 352.88049 ± 0.5, analogous to a quadrupole MS analysis) showed a wide, unresolved sample envelope that was caused by the transformer oil. Meanwhile, the high resolution EICC (*m/z* 352.88049 ± 0.05) eliminated the effect of the oil background, and extracted only the peaks for the penta-chlorinated PCBs.

[Conclusions]

These results demonstrate that the AccuTOF GC, a high resolution GC-TOFMS system, is a powerful tool for analyzing complex real-world samples that contain high levels of interfering impurities.

Determination of Triazolam by AccuTOF™ GC/Time-of-Flight Mass Spectrometry

Zhanpin Wu, JEOL USA, Inc.

Introduction

Triazolam is a benzodiazepine with a very short elimination half-life. The range is reported to be 1.5 to 5.5 hours[1]. Due to its frequent use as a sedative and its potential to affect human activities such as driving, an unambiguous and sensitive analysis method is needed for its identification and quantitation. Generally, for determination of triazolam, screen tests are performed in biological samples followed by confirmation and quantitation with GC/MS [2]. Here, we demonstrate the feasibility by using the JEOL AccuTOF™ GC, time-of-flight mass spectrometer with accurate mass measurement and negative ion chemical ionization (NCI) capabilities for triazolam determination. D_4-triazolam was used as internal standard. The mass accuracy without internal reference is smaller than 2 mmu. The limit of detection is 5 ng/mL. The quantitation standard curve can be linear from 5 ng/mL to 1000 ng/mL with R^2 of 0.9992. To the best of our knowledge, this is the first time that triazolam has been detected by GC/time-of-flight mass spectrometry with accurate mass measurement.

Experimental

1. Solvents and standards

All solvents used were of HPLC grade. Triazolam and d_4-triazolam standard solutions were purchased from Cerilliant (Round Rock, TX). A series of triazolam standard solutions, with concentrations from 5 ng/mL to 1000 ng/mL, was prepared in methanol. A stock solution of d_4-triazolam with 100 ng/mL was also prepared in methanol.

2. Sample preparation

An aliquot of 100 µL of sample was transferred into a small silanized glass tube, and then 100 µL of the internal standard was added. The solvent was evaporated under a gentle stream of nitrogen gas. Fifty microliters of ethyl acetate followed by 50 µL BSA/TMCS (5/1) were added to the tube. All tubes were heated at 80 °C for 30 min. The liquid was transferred to an autosampler vial for injection.

3. GC/MS analysis

The system included a JEOL AccuTOF™ GC time-of-flight mass spectrometry system set at NCI mode and an Agilent 6890 N GC. The system was controlled by a JEOL MassCenter™ workstation. The GC column was a DB5-MS capillary column (30 m, 0.25 mm i.d., 0.25µm). The initial oven temperature of 60 °C was held for 1 min, and then increased to 325 °C at the rate of 25 °C/min and held at the final temperature for 3 min. The carrier gas was helium with a constant flow rate of 1 mL/min. The temperatures for injection port, transfer line and ion source were 275 °C, 250 °C, and 200 °C, respectively. The reagent gas was methane with a flow rate of 0.83 mL/min. The ionizing voltage and current were 200 V and 300 μA, respectively. The MCP detector was set at 2,500 V. One microliter sample was injected onto the column with splitless mode.

Results and Discussion

Triazolam has very short elimination half-time. It is metabolized via hepatic microsomal oxidation. The hydroxylated metabolites, which are inactive, are excreted primarily in the urine as conjugated glucuronides. The level of parent drug in the biological fluid is usually very low after a few hours administration. It is generally known that negative-ion chemical ionization (NCI) provides very high sensitivity for analyzing compounds containing halogen atoms. Triazolam contains 2 chlorine atoms, making it possible to obtain very high sensitivity under NCI detection. Treatment with BSA/TMCS (5/1) improved the peak shapes of triazolam. Theoretically, only its hydroxylated metabolites – not the drug itself - should form TMS derivatives. However, TMS may enhance the chromatography for triazolam by either associating with the drug or by deactivating the GC column [3]. We first tested the detection limit under the current experimental conditions. An average signal-to-noise ratio of 70 was achieved when 5 ng/mL triazolam was injected. The error for mass accuracy is less than 2 mmu. Bigger than 3 mmu mass accuracy error was obtained if lower than 5 ng/mL samples were injected. Figure1 shows the high-resolution mass chromatogram (Δm = 0.01) and mass spectrum for m/z 306, $[M\text{-}Cl\text{-}H]^-$, in the standard solution with 5 ng/mL triazolam and 100 ng/mL internal standard.

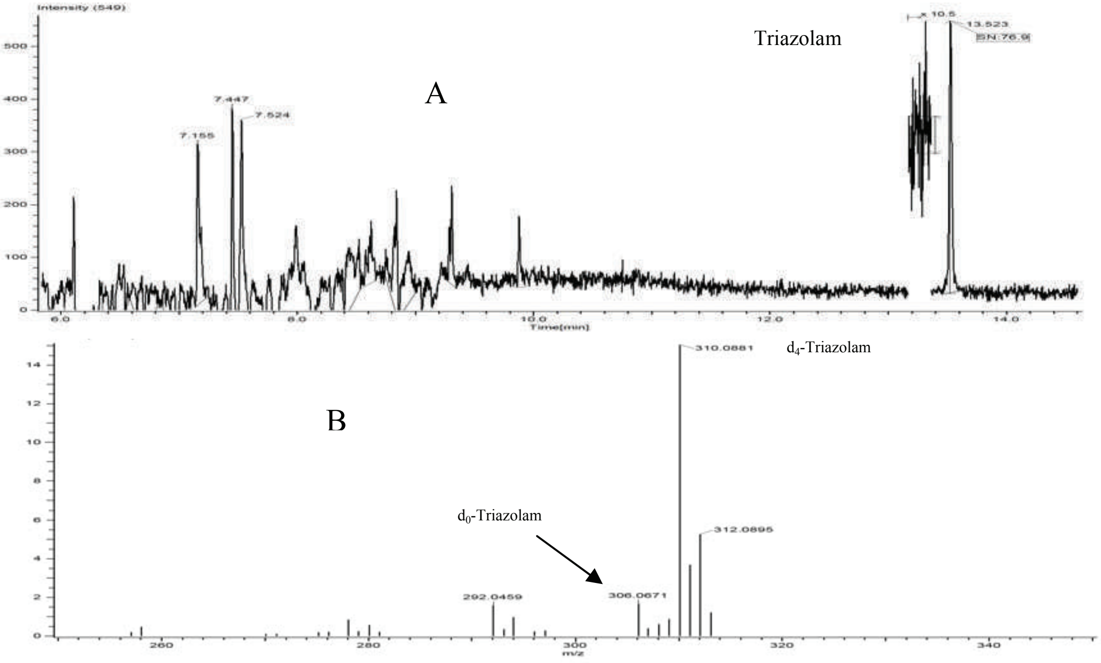

Fig.1 (A) Mass chromatogram of triazolam (m +/- 0.01) with concentration of 5 ng/mL. (B) Mass spectrum of traizolam with concentration of 5 ng/mL

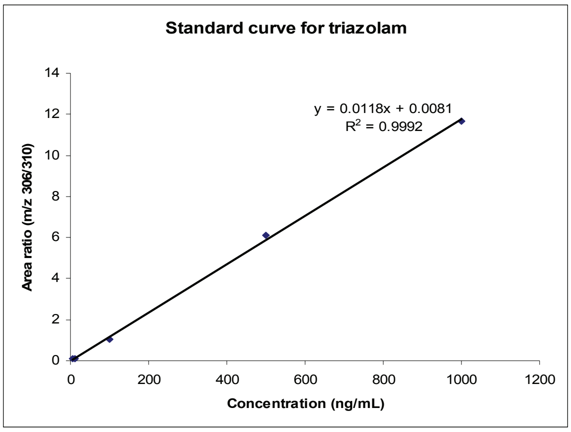

Fig. 2 Standard curve for triazolam from 5 ng/mL to 1000 ng/mL with d4-triazolam as the internal standard.

Concentration (ng/mL)	Precision % CV	Mass Accuracy (mmu)
5	16.2	1.44
10	3.2	1.12

Table 1. Precision and mass accuracy for two different concentrations of triazolam (n = 5)

Peak-area ratios (d_0/d_4) from high-resolution mass chromatograms were calculated for each standard and plotted against the known concentrations of the standard. Correlation coefficient (R^2) is 0.9992. The standard curve is shown in Figure 2.

The method precision was determined by analyzing two different concentrations of standard solution. The samples were analyzed five times in duplicate. The percent coefficient of variation (CV) is 3.2% for the 10 ng/mL sample and 16.2% for the 5 ng/mL sample. Higher than 20% CV was obtained if samples lower than 5 ng/mL were injected. Therefore, the quantitation limit for the assay is 5 ng/mL. The results are listed in Table 1.

Conclusion

To the best of our knowledge, this is the first time the feasibility of using GC/time-of-flight mass spectrometry to determine triazolam has been evaluated. The accurate mass measurement capability of a high-resolution time-of-flight mass spectrometer makes the determination unambiguous even in very low concentrations. The method was sensitive, precise and simple. In order to apply this method for biological samples, additional method development including sample extraction and validation may be required.

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AccuTOF™

A new method for pesticides identification: fast GC/time-of-flight mass spectrometry

Zhanpin Wu[1], Masaaki Ubukata[2], Akihiko Kusai[2], Rae Ann Baldwin[1]
1 JEOL USA, Inc. 11 Dearborn Road, Peabody, MA 01960
2 JEOL Limited, Tokyo, Japan

Introduction

Pesticides have been widely used all over the world. Although the use of pesticides is strictly regulated in many countries, laboratories still monitor their residues due to their toxicity and highly persistent nature. The most common method for pesticides identification is GC/MS with select ion monitoring (SIM). Since most of samples contain many different components, a long GC separation is generally needed when a low-resolution SIM MS is used. This is very time-consuming. Fast GC has been available for several years; however, the combination of fast GC with mass spectrometry had not been commercially available until high acquisition rate time-of-flight mass spectrometry was introduced. Here, we describe a new method by using fast GC/time-of-flight MS to identify 67 pesticides. The high resolution time-of-flight MS always yields high quality library searchable spectrums without compromising the sensitivity. The method is simple, fast, and reliable.

Experimental

All solvents used were of HPLC grade. The pesticide standards used are listed in Table 1. They were prepared in ethyl acetate with concentration of 100 ppb.

An Agilent 6890N gas chromatograph was used. Samples were injected onto an HP-5MS capillary column (10 x 0.18 mm, 0.18 μm film thickness) with splitless injection mode. Helium was used as the carrier gas and set at 0.6 mL/min. The injector temperature was set at 200 °C. The oven temperature was held at 40 °C for 1 min and then increased to 300 °C at a rate of 50 °C/min.

Pesticide	Formula	Pesticide	Formula	Pesticide	Formula
Simazine	C7H12ClN5	Thiobencarb	C12H16ClNOS	Isoxathion	C13H16NO4PS
Diazinon	C12H21N2O3PS	Fenitrothion	C9H12NO5PS	Isoprothiolane	C12H18N4S2
Chlorothalonil	C8Cl4N2	Propyzamide	C12H11Cl2NO	Dichlorvos	C4H7Cl2O4P
Fenobcarb	C12H17NO2	Chlornitrofen	C12H6Cl3NO3	CNP-amino	C12H8Cl3NO
Iprobenfos	C13H21O3PS	EPN	C14H14NO4PS	Isofenphos	C15H24NO4PS
Chlorpyrifos	C9H11Cl3NO3PS	Pyridafenthion	C14H17N2O4PS	Iprodione	C13H13Cl2N3O3
Etridiazole	C5H5Cl3N2OS	Captan	C9H8Cl3NO2S	Chloroneb	C8H8Cl2O2
Tolclofos-methyl	C9H11Cl2O3PS	Flutolanil	C17H16F3NO2	Pencycuron	C19H21ClN2O
Metalaxyl	C15H21NO4	Mepronil	C17H19NO2	Dithiopyr	C15H16F5NO2S2
Terbucarb	C17H27NO2	Napropamide	C17H21NO2	Pyributicarb	C18H22N2O2S
Butamifos	C13H21N2O4PS	Benfluralin	C13H16F3N3O4	Pendimethalin	C13H19N3O4
Methyldymron	C17H20N2O	Alachlor	C14H20ClNO2	Edifenphos	C14H15O2PS2
Pyroquilon	C11H11NO	Phthalide	C8H2Cl4O2	Mefenacet	C16H14N2O2S
Pretilachlor	C17H26ClNO2	Isoprocarb	C11H15NO2	Thenylchlor	C16H18ClNO2S
Methidathion	C6H11N2O4PS3	Bromobutide	C15H22BrNO	Molinate	C9H17NOS
Procymidone	C13H11Cl2NO2	Anilofos	C13H19ClNO3PS2	Atrazine	C18H14ClN5
Dichlobenil	C7H2Cl2N	Dimethoate	C5H12NO3PS2	Endosulphan	C9H6Cl6O3S
Etofenprox	C25H28O3	Fenthion	C10H15O3PS2	Malathion	C10H19O6PS2
Simetryne	C8H15N5S	Dimepiperate	C15H21NOS	Phenthoate	C12H17O4PS2
Buprofezin	C16H23N3OS	Ethyl thiometon	C8H19O2PS3	Esprocarb	85785-20-2
Bifenox	C14H9Cl2NO5	Piperophos	C14H28NO3PS2	Dimethametryn	C11H21N5S
Propiconazole	C15H17Cl2N3O2	Pyriproxyfen	C20H19NO3	Trifluralin	C13H16F3N3O4
Cafenstrole	C16H22N4O3S				

Table 1. List of 67 pesticides

The mass spectrometer system consisted of JEOL AccuTOF-GC™ time-of-flight mass spectrometer with EI source and JEOL MassCenter™ workstation. The source and transfer line temperature were set at 250°C, respectively. The detector voltage was set at 2500V. The acquisition range is from m/z 35 to 500 with spectrum recoding interval of 0.05 s. The system was tuned with PFK to achieve a resolution of 6,000 (FWHM) at m/z 292.9824.

Results

Figure 1 shows the TICs of 67 pesticides separated by the AccuTOF™ GC/MS system. The high acquisition rate of this GC/MS system makes fast GC separation possible. The running time is only 6.5 min.

In order to determine the data quality for the unresolved chromatographic peaks, we chose tolclofos-methyl and alachlor. Their retention times have only 0.008 min difference. Since time-of flight mass spectrometer always runs at high resolution and full mass range without compromising the sensitivity, a full mass-range spectrum can be obtained for each pesticide.

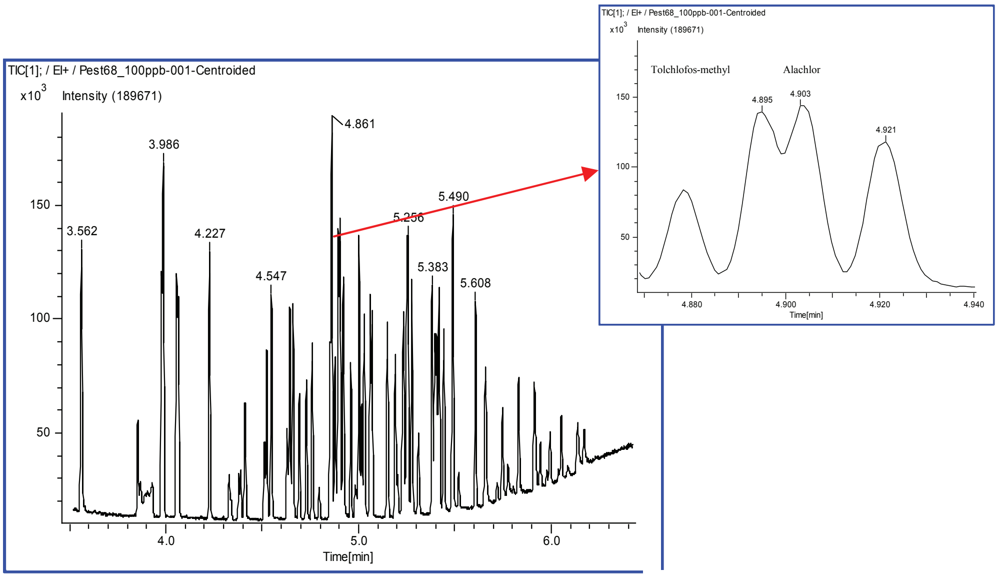


Fig 1. Total ion chromatogram of 67 pesticides.

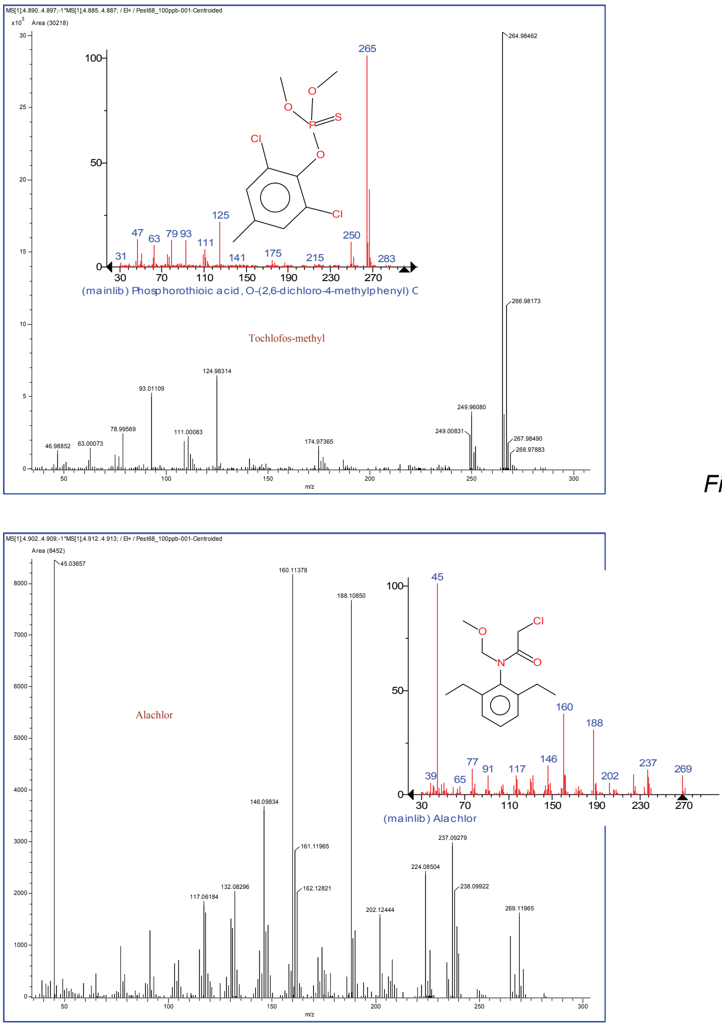
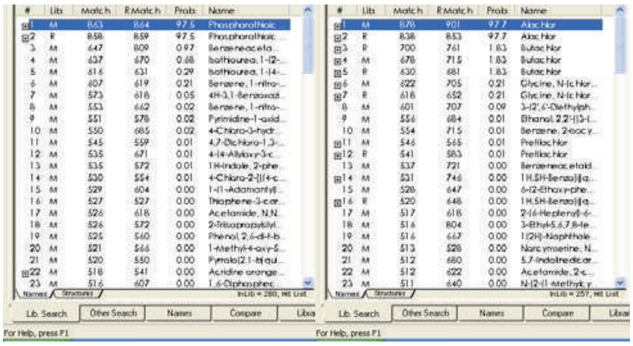


Fig. 2 Mass spectra for selected two pesticides

Figure 2 shows the mass spectra for these two pesticides in the sample and their corresponding spectra in the NIST library. An excellent library search was obtained and the results are shown in Figure 3. The probability index for both pesticides is greater than 97%, showing a strong confidence for identification results.

In addition, the high-resolution time-of-flight MS system has the capability for exact mass measurement. Possible elemental compositions for each pesticide and its fragments are ready obtained. The exact mass measurement results for two selected pesticides are listed in Table 2. The errors for all selected ions are less than 2 mmu.

The elemental composition estimation combined with full mass range spectrum make the identification unambiguous.



#	Lib	Match	R.Match	Prob	Name
1	M	863	864	97.5	Phosphorothioic ...
2	R	858	859	97.5	Phosphorothioic ...
3	M	647	809	0.97	Benzeneaceta...
4	M	637	670	0.68	Isothiourea, 1-(2-...
5	M	616	631	0.29	Isothiourea, 1-(4-...
6	M	607	619	0.21	Benzene, 1-nitro-...
7	M	573	618	0.05	4H-3,1-Benzoxazi...
8	M	553	662	0.02	Benzene, 1-nitro-...
9	M	551	578	0.02	Pyrimidine-1-oxid...
10	M	550	685	0.02	4-Chloro-3-hydr...
11	M	545	559	0.01	4,7-Dichloro-1,3-...
12	M	535	671	0.01	4-(4-Allyloxy-3-c...
13	M	535	572	0.01	1H-Indole, 2-phe...
14	M	530	554	0.01	4-Chloro-2-[[(4-c...
15	M	529	604	0.00	1-(1-Adamantyl)...
16	M	527	527	0.00	Thiophene-3-car...
17	M	526	618	0.00	Acetamide, N,N...
18	M	526	572	0.00	2-Triisopropylsilyl...
19	M	525	560	0.00	Phenol, 2,6-di-t-b...
20	M	521	566	0.00	1-Methyl-4-oxy-5...
21	M	520	550	0.00	Pyrrolo[2,1-b]qu...
22	M	518	541	0.00	Acridine orange...
23	M	516	607	0.00	1,6-Diphosphec...

#	Lib	Match	R.Match	Prob	Name
1	M	878	901	97.7	Alachlor
2	R	838	853	97.7	Alachlor
3	R	700	761	1.83	Butachlor
4	M	678	715	1.83	Butachlor
5	R	630	681	1.83	Butachlor
6	M	622	705	0.21	Glycine, N-(chlor...
7	R	618	652	0.21	Glycine, N-(chlor...
8	M	601	707	0.09	3-(2',6'-Diethylph...
9	M	556	684	0.01	Ethanol, 2,2'-[(3-(...
10	M	554	715	0.01	Benzene, 2-acy...
11	M	546	565	0.01	Pretilachlor
12	R	541	583	0.01	Pretilachlor
13	M	537	721	0.00	Benzeneacetald...
14	M	531	746	0.00	1H,5H-Benzo[ij]q...
15	M	528	647	0.00	6-(2-Ethoxy-phe...
16	R	520	648	0.00	1H,5H-Benzo[ij]q...
17	M	517	618	0.00	2-(6-Heptenyl)-6-...
18	M	516	804	0.00	3-Ethyl-5,6,7,8-te...
19	M	516	667	0.00	1(2H)-Naphthale...
20	M	513	528	0.00	Narcymoerine, N...
21	M	512	680	0.00	5,7-Indolinedicar...
22	M	512	622	0.00	Acetamide, 2-c...
23	M	511	640	0.00	N-[2-(1-Methylc...

Fig 3. NIST library research.

Tolclofos-methyl			
Measured m/z	Calc. m/z	Error (mmu)	Formula
124.9831	124.9826	0.5	$C_2H_6O_2PS$
174.9737	174.9717	2.0	$C_7H_5Cl_2O$
249.9608	249.9620	-1.2	$C_8H_8ClO_3PS$
264.9846	264.9855	-0.9	$C_9H_{11}ClO_3PS$
Alachlor			
146.0983	146.0970	1.3	$C_{10}H_{13}N$-H
160.1138	160.1126	1.2	$C_{11}H_{15}N$-H
174.0925	174.0919	0.6	$C_{11}H_{13}NO$-H
188.1085	188.1075	1.0	$C_{12}H_{15}NO$-H
202.1244	202.1232	1.2	$C_{13}H_{17}NO$-H
224.0850	224.0842	0.8	$C_{12}H_{15}ClNO$
237.0928	237.0920	0.8	$C_{13}H_{17}ClNO$
269.1197	269.1183	1.4	$C_{14}H_{20}ClNO_2$

Table 2. Exact mass measurement results for major fragment ions for two selected pesticides.

Conclusion

Fast GC/time-of-flight mass spectrometry was used to identify 67 pesticides in 6.5 minutes. Full mass range spectrum and exact mass measurement provide positive identification.

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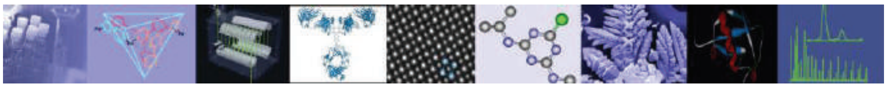
2. http://www.leco.org/customersupport/apps/separationscience/-227.pdf



AccuTOF-GC Series

Analysis of Electronics Waste by GCxGC Combined with High-resolution Mass Spectrometry: Using Accurate Mass Information and Mass Defect Analysis to Explore the Data [1]

Introduction

Comprehensive two-dimensional gas chromatography (GCxGC) in combination with high-resolution mass spectrometry (HRMS) is a powerful tool for the analysis of complex mixtures. However, new software tools are required to facilitate the interpretation of the rich information content in GCxGC/HRMS data sets. In this work, we analyzed a dust sample collected from an electronics recycling facility by using GCxGC in combination with a new high-resolution time-of-flight (TOF) mass spectrometer. Nontraditional Kendrick Mass Defect (KMD) plots were used to identify halogenated contaminants in an electronics waste sample. Database search results combined with elemental composition determinations from exact-mass data were used to identify (potential) persistent organic pollutants (POPs).

Sample and Instrument

A dust sample was collected from an electronics recycling facility. Then, 1 gram of this sample was used for extraction into hexane. Afterwards, the hexane solution was analyzed with the JEOL JMS-T100GCV “AccuTOF GCv 4G” equipped with a Zoex ZX2 thermal modulator (Figure 1) and a high-resolution version of the GC Image software (version 2.5.0a2). Table 1 shows the measurement conditions used for the analysis.

Result and Discussion

Figure 2a shows the GCxGC/EI TIC for the sample. Afterwards, a composite mass spectrum was created by summing the mass spectra for all components in the GC x GC/HRMS analysis (Figure 2b). Halogenated contaminants are readily recognized by their mass defects [2]. The next step then was to then create nontraditional KMD plots by converting the measured IUPAC *m/z* to H/Cl mass scales corresponding to the mass of a chlorine atom minus the mass of a hydrogen atom. Afterwards, the nominal mass was plotted vs. the corresponding mass defect for each peak (Figure 2c).

$$H/Cl\ mass = IUPAC\ mass \times (34/33.96102)$$

The resulting mass defect plot facilitated the rapid identification of families of compounds that differ by the number of chlorine substituents. The KMD plots for H/Cl and H/Br are nearly identical, allowing us to view both Cl and Br substitutions in one plot.

1. We found chlorinated and brominated compounds immediately, easily and visually by using H/Cl KMD plots. We then used this information to make the 2D mass chromatograms (Figure 3).
2. Additionally, we obtained NIST library search results and accurate mass measurement results from the data acquired in EI mode (Figure 3).
3. We showed a good example for the analysis of a complex sample using GCxGC, high-resolution MS and the KMD method. The combination of these techniques is a very powerful and useful tool for detailed qualitative analysis.
4. We also performed NICI measurements with GCxGC and KMD analysis (Figure 4). We observed similar results to the EI data for several compounds. However, some compounds showed abundant Cl^- and Br^- peaks instead of molecular ions so their 2D mass chromatograms were plotted as well (Figure 5). The NICI method is a good ionization technique for low-concentration samples and quantitative analysis.

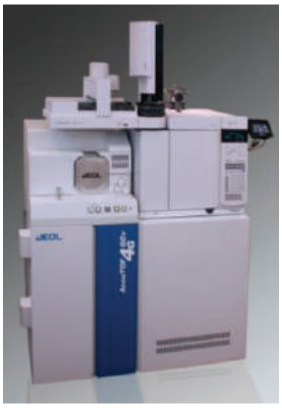


Figure 1. JMS-T100GCV AccuTOF GCv 4G GCxGC/HR-TOFMS system

Condition	GC x GC/EI	GC x GC/NICI
Sample	Dust sample collected from an electronics recycling facility	
GC x GC system	ZX2 thermal modulator (ZOEX)	
1st column	Rxi-5SilMS, 30 m x 0.25 mm, 0.25 µm	
2nd column	Rxi-17SilMS, 2 m x 0.15 mm, 0.15 µm	
Modulation loop	Deactivated fused silica, 1.5 m x 0.15 mm	
Modulation period	8 sec	
Modulation duration	400 msec	
Inlet pressure	200 kPa at Oven temp. 50 °C (Constant flow mode)	
Inlet mode	Splitless	
Oven temp.	50 °C (1 min) -> 5 °C/min -> 320 °C (5 min)	
GC-TOFMS system	AccuTOF GCv 4G (JEOL)	
Ionization mode	EI+	CI-
Ionization voltage	70 V	150 V
Ionization current	300 µA	300 µA
CI gas		Ammonia/Methane 0.5 mL/min
Ion source temp.	250 °C	200 °C
GC-ITF temp.	280 °C	
m/z range	*m/z* 45-800	*m/z* 30-800
Acquisition time	20 msec (50 Hz)	
Sampling time	0.25 nsec (4 GHz)	
External calibrant	*m/z* 207.0329 ($C_5H_{15}O_3Si_3^+$)	*m/z* 234.9405 (ReO_3^-)
Software	GC Image™ Version 2.5.0a2	

Table 1.Measurement Conditions

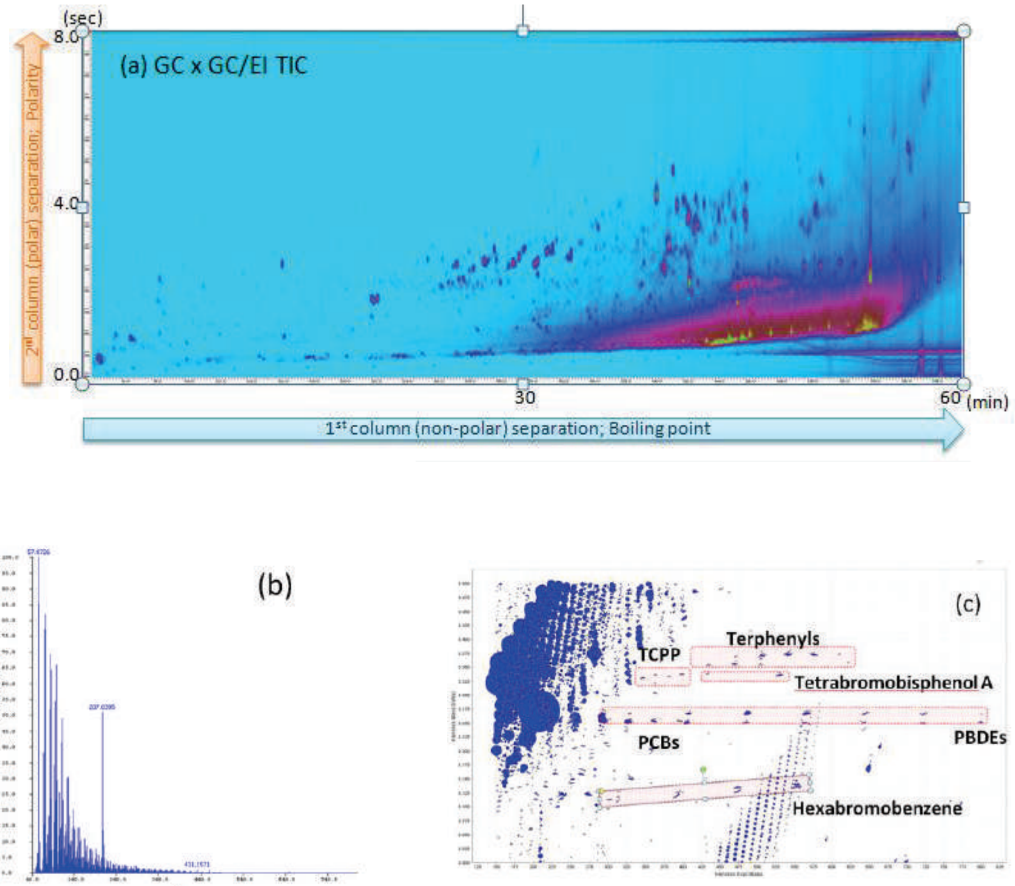


Figure 2. (a) GCxGC/EI TIC chromatogram of the dust sample, (b) Averaged mass spectrum for the whole retention time region, (c) H/Cl mass defect plot for the averaged mass spectrum.

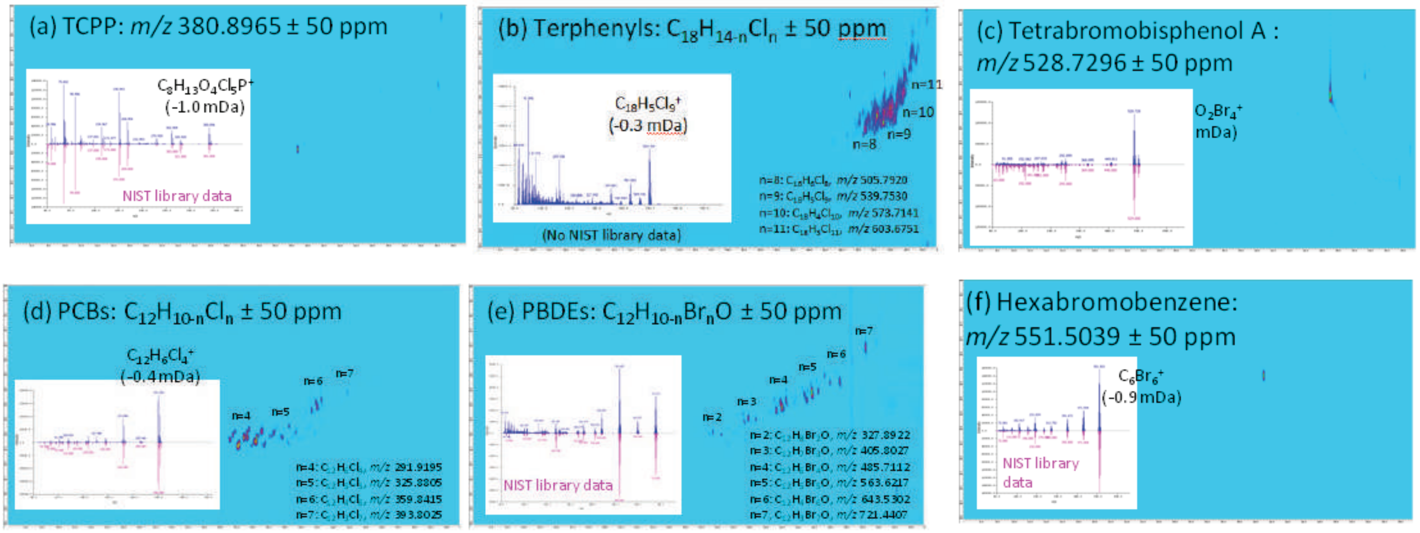


Figure 3. 2D mass chromatogram using mass of the most abundant isotope ion ± 50 ppm (a) TCPP, (b) Terphenyls, (c) Tetrabromobisphenol A, (d) PCBs, (e) PBDEs, (f) Hexabromobenzene

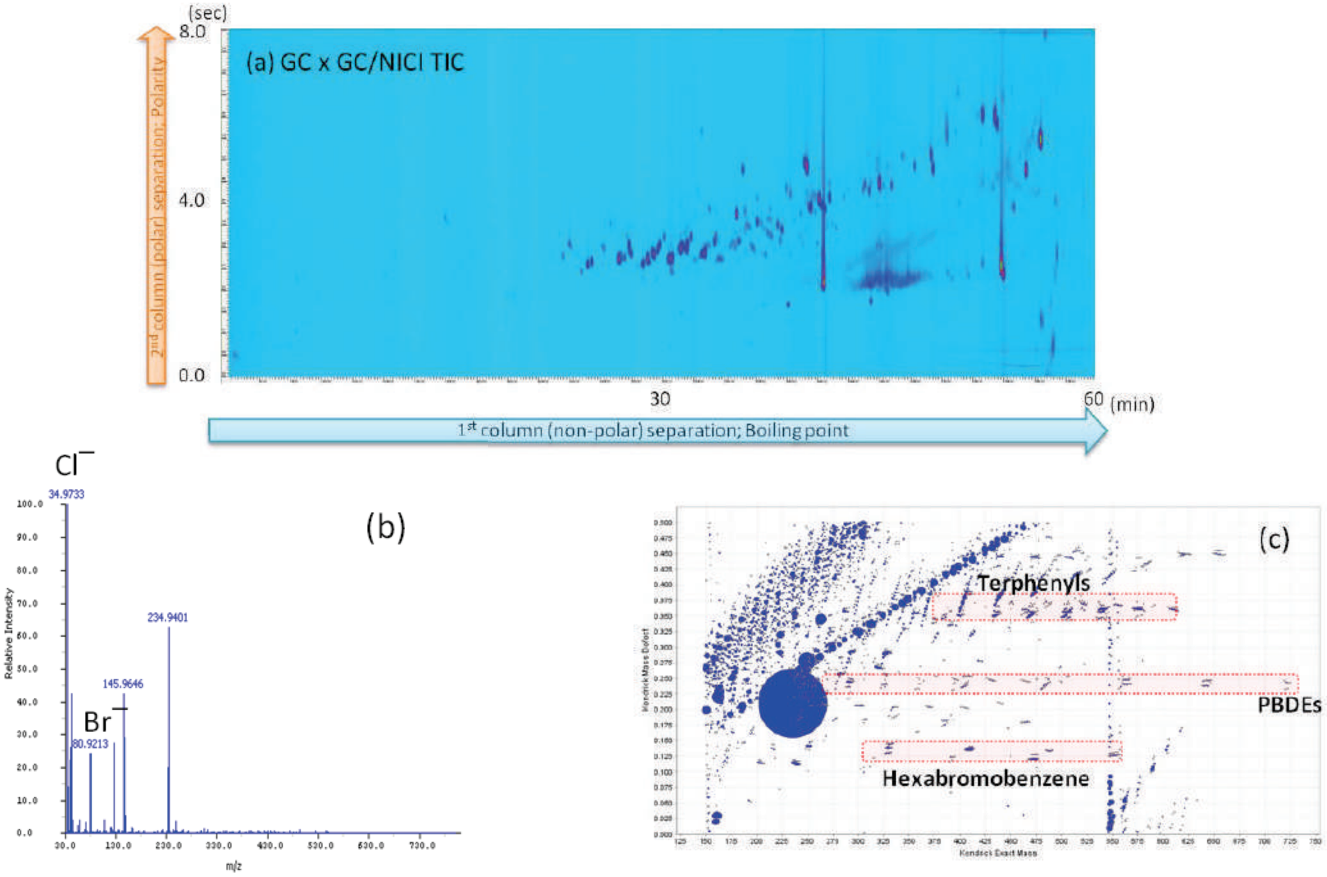


Figure 4. (a) GCxGC/NICI TIC chromatogram of the dust sample, (b) Averaged mass spectrum for the whole retention time region, (c) H/Cl mass defect plot for the averaged mass spectrum.

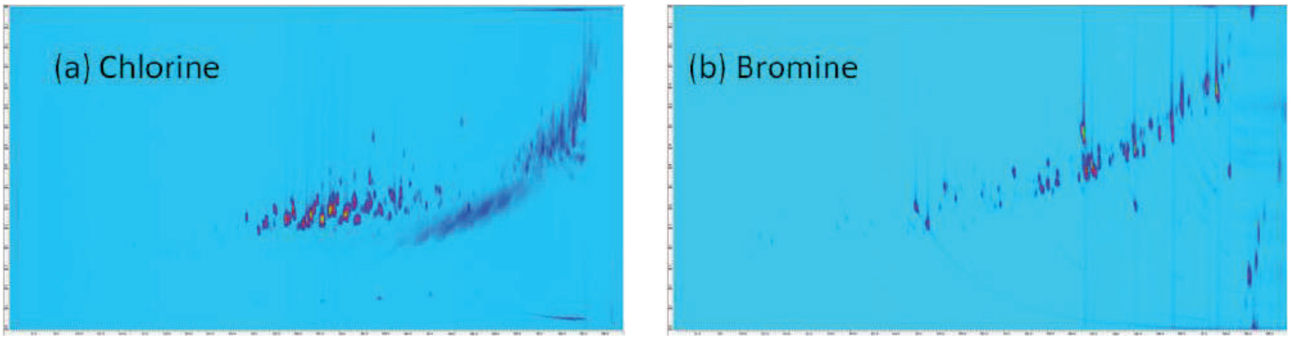
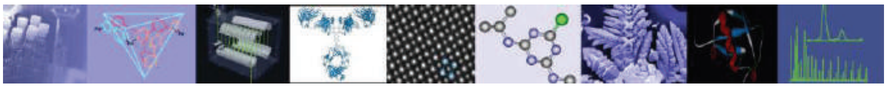


Figure 5. 2D mass chromatogram using mass of the most abundant isotope ion ± 0.02 u for (a) Chlorine (m/z 34.9689), and (b) Bromine (m/z 78.9183)

Reference

[1] M. Ubukata, K. J. Jobst, E. J. Reiner, S. E. Reichenbach, Q. Tao, J. Hang, Z. Wu, A. J. Dane, R. B. Cody. Non-targeted analysis of electronics waste by comprehensive two-dimensional gas chromatography combined with high-resolution mass spectrometry: using accurate mass information and mass defect analysis to explore the data. *Journal of Chromatography A*, **2015**. doi:10.1016/j.chroma.2015.03.050

[2] K. J. Jobst, L. Shen, E. J. Reiner, V. Y. Taguchi, P. A. Helm, R. McCrindle, S. Backus. The use of mass defect plots for the identification of (novel) halogenated contaminants in the environment, *Anal Bioanal Chem (2013)* 405:3289–3297



AccuTOF-GCv Series

Analyzing Polycyclic Aromatic Hydrocarbons in Diesel Particulate Matter using GC×GC-HRTOFMS

Introduction

Polycyclic aromatic hydrocarbons (PAHs) refer to aromatic hydrocarbons that have at least 2 benzene rings, such as naphthalene and anthracene which feature 2 and 3 benzene rings, respectively. PAHs are found in crude oil and are often released into the environment (water, atmosphere, etc.) when crude oil and oil products such as heating oil and light gas oil are burned. As it turns out most PAHs are carcinogenic with some of them identified as highly carcinogenic. Therefore, it is critical to determine the levels of PAHs present in the environment. In this work, we analyzed PAHs in diesel particulate matter using GCxGC-HRTOFMS, a technique that combines a JMS-T100GCV with comprehensive 2DGC (GCxGC).

Experimental

A commercial sample (2975 Diesel Particulate Matter, NIST) was mixed with chloroform (weight ratio 1:10) and heated at 100°C for 5 hours to accomplish solvent extraction. The extract was then centrifugally separated from the particulate material, and the resulting supernatant liquid was concentrated for measurement. Table 1 shows the instrument measurement conditions used for the analysis.

Instrument	JMS-T100GCV (JEOL Ltd.)
	KT2004 (Zoex Corporation)
Injection mode	Split 20:1
Injection temp.	300℃
Oven temp. program	50℃ (1min)→5℃/min→280℃(13min)
Injection volume	0.2μL
Column	1st: DB-1ms、30m×0.25mm、0.25μm
	2nd: DB-17、2m×0.1mm、0.1μm
Carrier gas	He、2.0 mL/min、Constant flow
Modulation	Period: 6sec、Releasing: 0.3sec
Ionization mode	EI+、70eV、300μA
Ion source temp.	280℃
m/z range	*m/z* 29－800
Data acquisition speed	0.04 sec(25 Hz)

Table 1. GCxGCHRTOF-MS measurement conditions

Results and Discussion

Figure 1 shows the GCxGC TIC chromatogram acquired from the sample. The X axis represents analyte separation by the 1st column, DB-1ms, in which the components

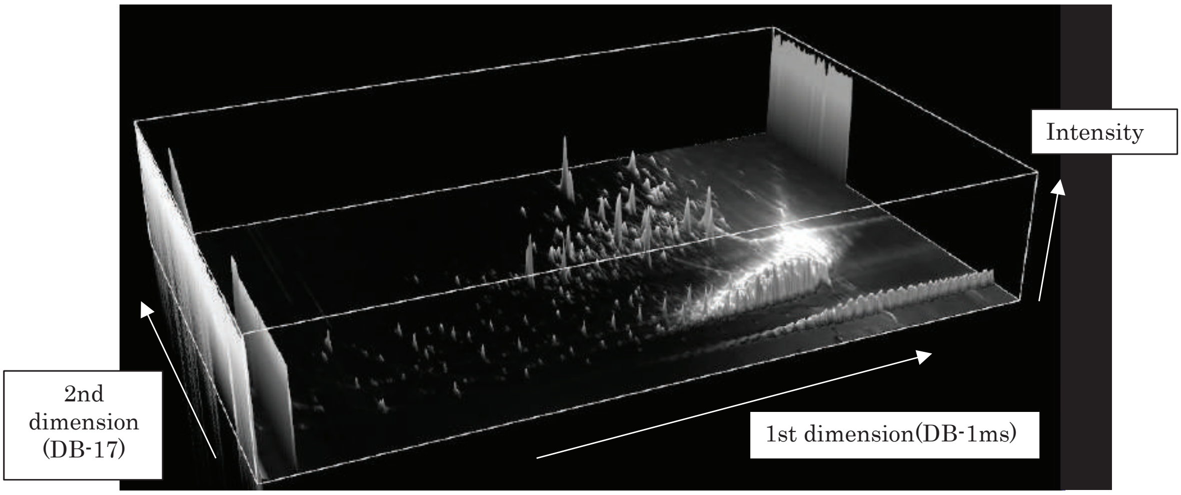


Figure 1. GCxGC TIC chromatogram (3D)

were separated according to their boiling points. The Y axis represents the separation in the 2nd column, DB-17, in which the components were separated according to their polarities. The Z axis represents the peak intensity of the ions in the TIC. Although the sample preparation consisted of a simple solvent extraction of the commercial sample, a wide variety of components were observed in the TIC (Figure 1).

Next, several mass chromatograms for $C_{18}H_{12}$, which contains 4 fused benzene rings, were compared to examine the selective detection of PAHs. The wide *m/z* window GCxGC mass chromatogram in Figure 2 (a) shows a number of impurities in addition to the PAHs. However, in the narrow *m/z* window GCxGC mass chromatogram in Figure 2 (b), only the PAHs were selectively detected. Figure 3 shows the mass spectrum for the PAHs detected in Figure 2. The mass spectrum shows that $C_{18}H_{12}$ (*m/z* 228.0930), a molecular ion of PAHs, was detected with high mass accuracy (0.3 mDa).

Conclusions

The results show that the GCxGC/HRTOFMS enhances the resolution of the GCxGC and the selectivity of HRTOFMS. Furthermore, combining these techniques is a powerful tool for analyzing complex samples that consist of many components and impurities.

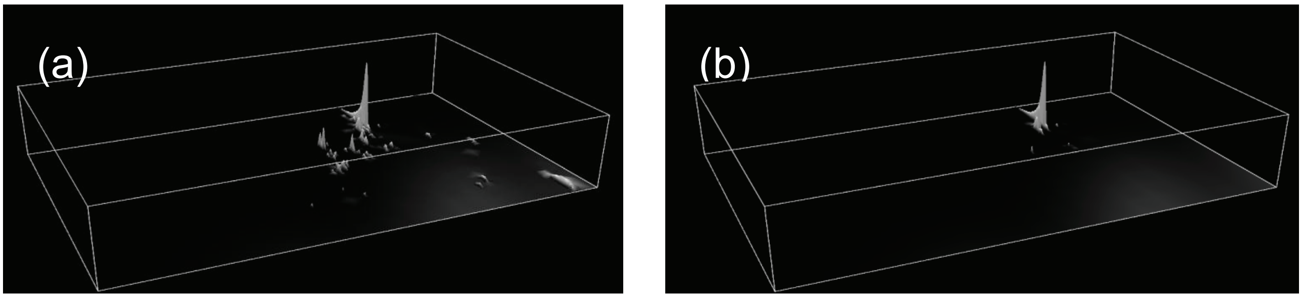


Figure 2. GCxGC chromatograms (3D)
(a) m/z 228±0.5 (b) 228.0930±0.01

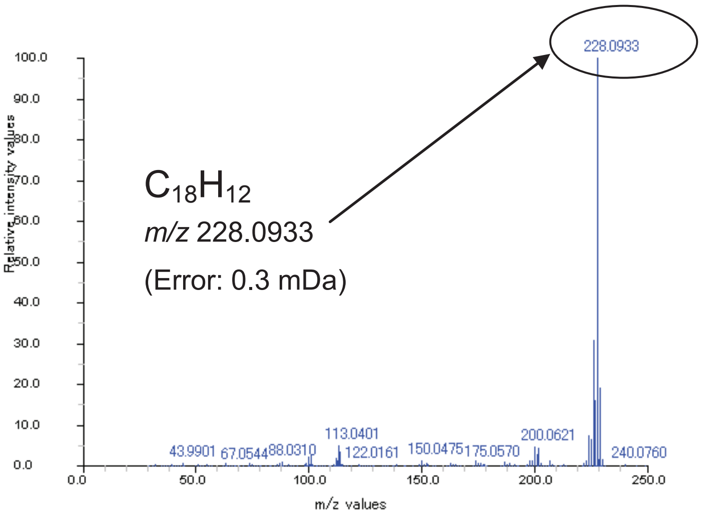


Figure 3. EI mass spectrum for $C_{18}H_{12}$ in Figure 2 (a) and (b)

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