C146-E260A

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Excellence in Science

Gas Chromatograph Mass Spectrometer

GCMS-TQ8040 Solutions

Triple Quadrupole Gas Chromatograph Mass Spectrometer

GCMS-TQ8040

So ***Smart*** almost runs itself!

Smart
Productivity
Operation
Performance

Finally, a triple quadrupole GCMS Smart enough for everyday use in your laboratory!

Smart Productivity

- Retention time synchronized MRM provides simultaneous sensitivity and precision for hundreds of compounds in one run
- Smart MRM optimizes analysis of 400+ compounds in a single acquisition with maximum sensitivity
- Reduced downtimes with the Twin Line configuration MS system

Smart Operation

- MRM Optimization tool to optimize MRM transitions automatically
- Smart Database Series with fully optimized transitions for hundreds compounds
- Smart MRM for automatic method creation in a single step

Smart Performance

- The patented ion source design and uniform temperature prevent active spots and boost sensitivity
- The OFF-AXIS ion optics eliminate chemical noise and lower detection limits
- High-sensitivity analysis even in single GC-MS mode

Food

Environment

Life Science

Forensics

Investigation of Simultaneous Analysis Methods for 420 Residual Pesticide Compounds

Due to its excellent sensitivity and selectivity, GC-MS/MS is utilized for the analysis of residual pesticides in foods. The number of relevant pesticides grows yearly, and has reached 300 to 400 compounds. Due to limitations in MRM-related software functionality and detection sensitivity, analyzing so many pesticides requires the intended pesticides to be divided into multiple method files and then measured method by method. As a result, the number of analyses increases, and places a strain on laboratory productivity. Smart MRM, the method creation function in GCMSsolution software, automatically creates the optimal methods for the simultaneous analysis of over 400 compounds, while ensuring sensitivity and accuracy are maintained. In addition, MRM analysis can be started without configuring troublesome transition settings by utilizing Smart Pesticides Database, which contains acquisition parameters for 479 compounds.
Batch analysis methods for 420 compounds (approximately 1,200 transitions) using Smart Pesticides Database and Smart MRM is reported here.

GCMS-TQ8040 Features

- New firmware protocol enables MRM measurements with up to 32,768 transitions in a single analysis.
- Automatic creation of optimal methods by Smart MRM

Smart Pesticides Database Features

- Includes compound information for approximately 480 components and supports simultaneous analysis by GC-MS/MS
- Retention time correction using retention indices (AART function)

Experiment

Commercially available spinach was pretreated with the QuEChERS method using Restek Q-sep™. Pesticides were added to the sample extract, with the concentration adjusted to 5 ng/mL. The prepared sample was then subjected to MRM analysis for 420 compounds under the analysis conditions registered in Smart Pesticides Database. Table 1 shows the analysis conditions.
The retention times for the individual pesticides were corrected using the AART function based on an *n*-alkane analysis.

Table 1: Analysis Conditions

GC-MS	GCMS-TQ8040
Column	SH-Rxi™-5Sil MS (Length 30 m, 0.25 mm I.D., df = 0.25 µm) (Shimadzu, P/N: 221-75954-30)
Glass Insert	Sky™ Liner, Splitless Single Taper Gooseneck w/Wool (Restek, P/N: 567366)
[GC]	
Injection Port Temp.	250 °C
Column Oven Temp.	50 °C (1 min.) → (25 °C/min.) → 125 °C (10 °C/min.) → 300 °C (15 min.)
Injection Mode	Splitless
High-Pressure Injection	250 kPa (1.5 min)
Carrier Gas Control	Linear velocity (47.2 cm/sec.)
Injection Volume	2 µL
[MS]	
Interface Temp.	250 °C
Ion Source Temp.	200 °C
Solvent Elution Time	1.5 min.
Acquisition Mode	MRM
Loop Time	0.5 sec.

Analysis Results

Fig. 1 shows the mass chromatograms for malathion, trifloxystrobin, and fenbuconazole. Table 2 shows the area repeatability values for 240 of the 420 compounds (n = 5). By creating suitable MRM analysis methods utilizing Smart MRM, it is possible to analyze even 420 compounds simultaneously with favorable sensitivity and accuracy.

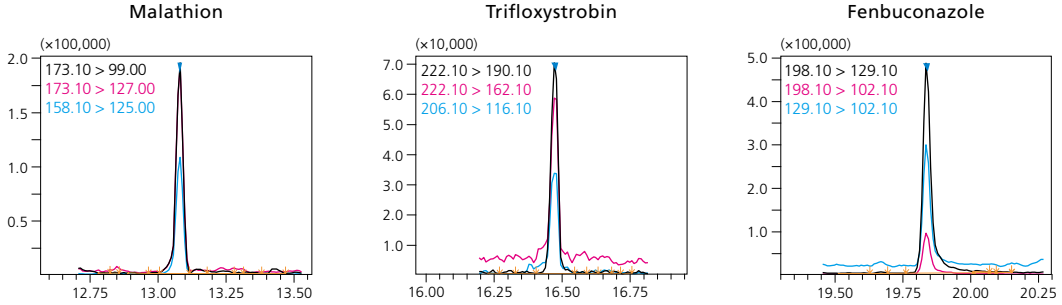


Fig. 1: Mass Chromatograms for the Pesticides

Table 2: Area Repeatability for 240 Compounds (n = 5)

Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD
Dichlorvos	6.37	Pyrimethanil	3.55	Fosthiazate-1	5.22	Carboxin	3.94	Fenamidone	2.31
Dichlobenil	2.87	Isazofos	3.89	Fosthiazate-2	8.17	Diclobutrazol	4.21	Tebufenpyrad	6.29
EPTC	2.28	Tefluthrin	2.58	Pendimethalin	6.83	(Z)-Metominostrobin	6.00	Bifenox	7.37
Butylate	2.55	Terbacil	6.73	(E)-Chlorfenvinphos	3.27	Azaconazole	2.75	Furametpyr	4.71
Etridiazole	8.94	Etrimfos	3.20	Cyprodinil	4.35	Cyflufenamid	9.37	Tetradifon	7.80
Methacrifos	3.84	delta-BHC	7.35	Fipronil	6.31	Chlorfenapyr	7.41	Pentoxazone	5.03
Clothianidin	9.96	Tri-allate	4.62	Dimethametryn	2.44	Isoxathion	9.27	Phosalone	8.42
Chloroneb	3.36	Tebupirimfos	3.79	Penconazole	4.01	(Z)-Pyriminobac-methyl	2.59	Leptophos	4.31
Crimidine	3.31	Iprobenfos	2.12	Chlozolinate	10.85	Chlorobenzilate	1.33	Azinphos-methyl	4.17
2-Phenylphenol	2.74	Benoxacor	9.77	Tolylfluanid	5.13	Fensulfothion	6.20	Cyhalothrin-1	9.01
Isoprocarb	4.68	Dichlofenthion	2.62	Isofenphos	2.82	beta-Endosulfan	7.09	Cyhalothrin-2	8.68
Tecnazene	3.66	Dimethenamid	2.55	Phenthoate	3.43	Diniconazole	3.46	Cyhalofop-butyl	1.48
Omethoate	6.96	Propanil	1.78	Quinalphos	7.29	Oxadixyl	4.14	Mefenacet	4.52
Propoxur	9.13	Acetochlor	3.83	Thiabendazole	3.55	Ethion	3.10	Pyrazophos	5.85
Propachlor	2.73	Bromobutide	4.15	Dimepiperate	2.29	Fluacrypyrim	3.59	Fenarimol	4.05
Ethoprophos	2.55	Chlorpyrifos-methyl	2.91	Procymidone	1.15	Mepronil	1.35	Azinphos-ethyl	3.58
Ethalfluralin	3.83	Vinclozolin	3.91	Bromophos-ethyl	1.78	Triazophos	6.34	Pyraclofos	7.94
Chlorpropham	3.21	Parathion-methyl	6.53	Methidathion	2.84	Chlornitrofen	5.56	Fenoxaprop-ethyl	9.11
Trifluralin	5.56	Tolclofos-methyl	2.70	Chlorbenside	3.44	Carbophenothion	3.72	Fluquinconazole	11.11
Dicrotophos	5.78	Simeconazole	4.94	Propaphos	7.33	Cyanofenphos	4.13	Pyridaben	5.75
Benfluralin	5.09	Alachlor	4.17	Tetrachlorvinphos	6.45	Trifloxystrobin	2.52	Butafenacil	4.30
Salithion	2.10	Simetryn	3.24	Trichlamide	13.53	Edifenphos	7.31	Etobenzanid	2.99
Sulfotep	3.61	Metalaxyl	2.51	Paclobutrazol	3.63	Norflurazon	4.58	Fenbuconazole	2.93
Monocrotophos	5.44	Fenchlorphos	2.94	Butachlor	4.03	Propiconazole-1	7.77	Cypermethrin-1	14.35
Cadusafos	3.25	Prometryn	4.12	Fenothiocarb	2.83	Propiconazole-2	7.45	Cypermethrin-2	9.04
Phorate	1.99	Pirimiphos-methyl	3.14	alpha-Endosulfan	13.10	Quinoxyfen	2.34	Cypermethrin-3	9.50
alpha-BHC	4.14	Fenitrothion	4.47	Butamifos	4.96	(E)-Pyriminobac-methyl	0.69	Cypermethrin-4	9.03
Thiometon	3.57	Ethofumesate	4.15	Flutriafol	5.72	Endosulfan sulfate	12.96	Halfenprox	4.03
Dicloran	6.15	(E)-Dimethylvinphos	4.82	Fenamiphos	6.49	Lenacil	2.35	Flucythrinate-1	7.57
Dimethoate	5.51	Bromacil	7.35	Napropamide	8.75	Chloridazon	7.76	Flucythrinate-2	7.78
Furilazole	2.52	Esprocarb	3.06	Flutolanil	6.05	Tebuconazole	5.87	Quizalofop-ethyl	5.45
Carbofuran	8.29	Malathion	7.44	Hexaconazole	8.09	Piperonyl butoxide	3.48	Etofenprox	4.39
Simazine	4.30	Quinoclamine	6.49	Prothiofos	3.47	Epoxiconazole	0.42	Silafluofen	1.71
Atrazine	1.34	Metolachlor	1.79	Fludioxonil	2.39	Zoxamide	7.71	Fluridone	2.51
Dimethipin	8.14	Chlorpyrifos	3.63	Isoprothiolane	4.10	Pyributicarb	5.85	Pyrimidifen	4.83
Swep	3.23	Thiobencarb	7.20	Pretilachlor	3.01	Chlomethoxyfen	4.34	Flumioxazin	13.53
beta-BHC	1.62	(Z)-Dimethylvinphos	2.82	Profenofos	2.96	Pyridaphenthion	3.17	Fenvalerate-1	7.70
Chlorbufam	7.21	Diethofencarb	2.10	Tricyclazole	5.31	Iprodione	8.59	Fenvalerate-2	5.06
Clomazone	3.10	Fenthion	6.45	Uniconazole	3.63	Acetamiprid	7.64	Pyraclostrobin	3.40
Quintozene	2.85	Chlorthal-dimethyl	3.06	Oxadiazon	2.86	Phosmet	10.86	Difenoconazole-1	9.02
Propazine	6.04	Fenpropimorph	4.62	Thifluzamide	5.38	Bifenthrin	6.12	Difenoconazole-2	3.94
gamma-BHC	4.52	Parathion	8.92	Tribufos	2.04	EPN	7.46	Indoxacarb	13.62
Terbufos	2.88	Triadimefon	3.61	Myclobutanil	2.62	Bromopropylate	3.29	Azoxystrobin	8.90
Cyanophos	4.00	Tetraconazole	6.35	Flusilazole	6.76	Picolinafen	3.06	Dimethomorph-1	6.48
Fonofos	5.74	Isocarbophos	8.27	Oxyfluorfen	12.31	Fenoxycarb	5.28	Dimethomorph-2	7.81
Propyzamide	1.97	Nitrothal-isopropyl	6.41	Bupirimate	3.78	Bifenazate	3.52	Tolfenpyrad	5.71
Pyroquilon	4.53	Phthalide	5.64	Buprofezin	5.30	Etoxazole	7.00	Imibenconazole	7.03
Diazinon	3.33	Bromophos	3.73	Kresoxim-methyl	2.86	Fenpropathrin	6.37	Cinidon-ethyl	10.40

Easy Screening for Residual Pesticides in Foods

The analysis of residual pesticides in processed foods using GC-MS/MS, which provides excellent selectivity and sensitivity, has become a focus of attention.
Before starting GC-MS/MS measurements, it is necessary to optimize MRM transitions (precursor ions and product ions) and collision energies (CE) for each pesticide measured, which is extremely labor intensive. Furthermore, in order to calculate quantitative values, it is necessary to prepare standard samples and create calibration curves.
The Quick-DB database contains the optimal MRM conditions (MRM transitions and CE), mass spectra, retention indices, calibration curves and other information. This enables the semi-quantitative analysis of pesticides without using standard samples. Pesticide surrogates are used as the internal standard substances for calibration curves. Favorable quantitative accuracy is achieved by selecting the surrogates suited to each pesticide.
In analyzing residual pesticides in processed foods, which contain a number of contaminants, separating the pesticides from the contaminants can be impossible, even with GC-MS/MS. In this case, an effective approach to separating and detecting the pesticides is to perform the analysis with two columns respectively, which differ in their separation patterns. The information registered in Quick-DB is also compatible with analysis using two different columns for residual pesticides in processed foods. In addition, if the Twin Line MS system is used, the two columns can be attached to the MS unit simultaneously, so data can be sampled from the different columns smoothly, without compromising the MS vacuum. Here is the application data reports on the results of applying Quick-DB and the Twin Line MS system to the analysis of residual pesticides in ginger.

GCMS-TQ8040 Features

- Simultaneous Scan/MRM analysis using Advanced Scanning Speed Protocol (ASSP™)
- Twin Line MS system simplifies column replacement.

Quick-DB "GC/MS Residual Pesticide Database" Features

- Quantitative analysis without standard samples
- Supports the Twin Line MS system for analysis of high matrix samples

Experiment

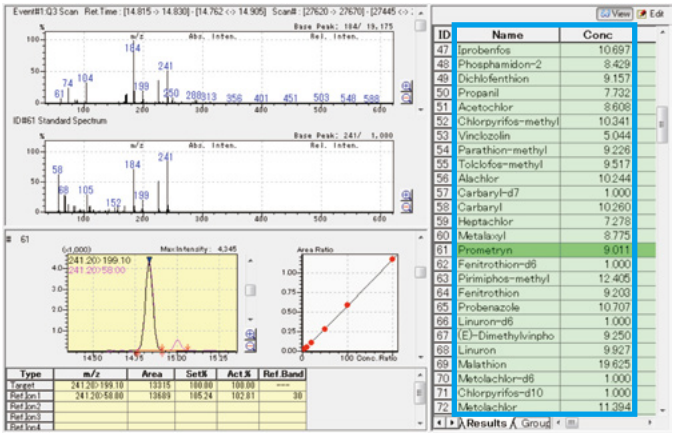
Using the Restek Q-sep™, ginger was pretreated via the QuEChERS method. The sample solution obtained was spiked with 230 standard pesticide samples at a concentration of 10 ng/mL. The pesticide-spiked samples were then subjected to Scan/MRM analysis under the analysis conditions registered in Quick-DB. The analysis conditions are shown in Table 1. The two columns indicated in Table 1 were installed to a single GC-MS with the Twin Line MS system. The retention times for the pesticide components were estimated based on the analysis results for the *n*-alkane standard sample.

Table 1: Analysis Conditions

GC-MS	GCMS-TQ8040 (Twin Line MS System)
Column 1	SH-Rxi™-5Sil MS (Length 30 m, 0.25 mm I.D., df=0.25 µm) (Shimadzu, P/N: 221-75954-30)
Column 2	SH-Rtx™-200MS (Length 30 m, 0.25 mm I.D., df=0.25 µm) (Shimadzu, P/N: 221-75811-30)
Glass Insert	Sky™Liner, Splitless Single Taper Gooseneck w/Wool (Restek, P/N: 567366)
[GC]	
Injection Port Temp.	250 °C
Column Oven Temp.	60 °C (1 min.) → (25 °C /min.) → 160 °C → (4 °C /min.) → 240 °C → (10 °C /min.) → 290 °C (11 min.)
Injection Mode	Splitless
High-Pressure Injection	250 kPa (1.5 min.)
Carrier Gas Control	Linear velocity (40.0 cm/sec.)
Injection Volume	2 µL
[MS]	
Interface Temp.	300 °C
Ion Source Temp.	200 °C
Solvent Elution Time	1.5 min.
Acquisition Mode	FAAST (Scan/MRM simultaneous measurement)
Scan Mass Range	*m/z* 50 to 600
Scan Event Time	0.15 sec.
Scan Speed	5,000 u/sec.

Analysis Results

The liquid food extract spiked with pesticides was analyzed, and data processing was performed with Quick-DB. The analysis results are shown in Fig. 1. When semi-quantitative analysis was performed using the calibration curves registered in Quick-DB, favorable semi-quantitative values were obtained, close to the additive concentration of 10 ng/mL for many of the components.
To evaluate the quantitative accuracy for this analysis method, ratios were calculated for the semi-quantitative values with respect to the additive concentration. Then the pesticides were classified into those with a ratio under 50 %, 50 % to 200 %, and over 200 %, to find the distribution. The results are shown in Fig. 2. A significant 75 % of components had a semi-quantitative value 50 % to 200 % that of the concentration of the standard pesticide samples added. From this, it is evident that semi-quantitative analysis can be performed with high accuracy.



ID	Name	Conc
47	Iprobenfos	10.697
48	Phosphamidon-2	8.429
49	Dichlofenthion	9.157
50	Propanil	7.732
51	Acetochlor	8.608
52	Chlorpyrifos-methyl	10.341
53	Vinclozolin	5.044
54	Parathion-methyl	9.226
55	Tolclofos-methyl	9.517
56	Alachlor	10.244
57	Carbaryl-d7	1.000
58	Carbaryl	10.260
59	Heptachlor	7.278
60	Metalaxyl	8.775
61	Prometryn	9.011
62	Fenitrothion-d6	1.000
63	Pirimiphos-methyl	12.405
64	Fenitrothion	9.203
65	Probenazole	10.707
66	Linuron-d6	1.000
67	(E)-Dimethylvinpho	9.250
68	Linuron	9.927
69	Malathion	19.625
70	Metolachlor-d6	1.000
71	Chlorpyrifos-d10	1.000
72	Metolachlor	11.394

As a result of calculation of the semi-quantitative values using the calibration curves preregistered in Quick-DB, favorable quantitative values were obtained.
Fig. 1: Analysis Results for the Pesticide-Spiked Samples (10 ng/mL concentration)

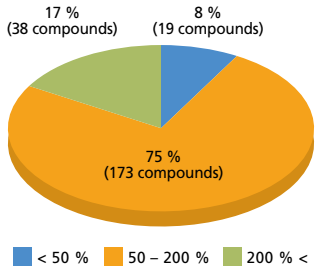


Fig. 2: Percentage Distribution of Semi-Quantitative Values with Respect to the Additive Concentration
(For 14 components, Rtx-200MS data are used.)

In the analysis of residual pesticides in foods, when pesticide peaks are detected, it is necessary to check whether contaminants have been misidentified as pesticides, and whether contaminant overlap has inflated the size of the quantitative values.
One confirmation method is to analyze the samples with columns with different separation patterns, and then check that essentially the same quantitative values are obtained for the pesticides detected in the respective columns. As an example, Fig. 3 shows the analysis results for methamidophos. Even for pesticides of which separation from contaminants is difficult, separation is possible if using columns with different separation patterns, enabling highly reliable semi-quantitative analysis.

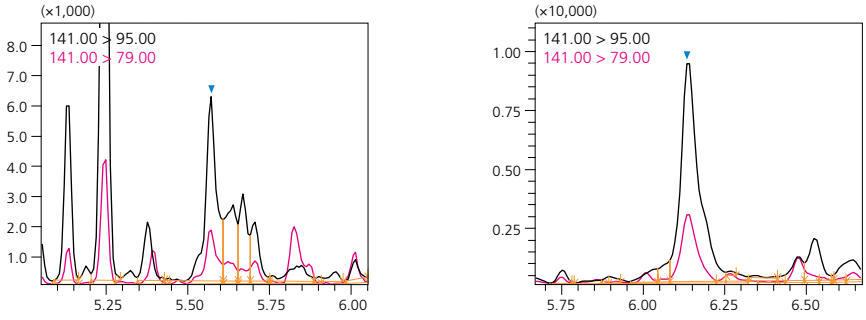


Fig. 3: Chromatograms for Ginger Extract, Spiked with Methamidophos (10 ng/mL Concentration)
(Left: Rxi-5Sil MS; Right: Rtx-200MS)

High-accuracy semi-quantitative analysis was achieved quickly and easily, by attaching two columns to the GCMS-TQ8040 utilizing the Twin Line MS system, and then screening for residual pesticides in foods using Quick-DB.

Analysis of PCBs and Organochlorinated Pesticides in River Water Using Simultaneous Scan/MRM Measurement

MRM analysis using GC-MS/MS is an effective way to measure trace environmental pollutants. However, in recent years incidents of chemical spills have increased worldwide and in order to investigate the cause and respond quickly, demand for the identification of unanticipated chemicals has heightened. As a result, analyses that target as many chemicals as possible and that can identify compounds from mass spectrum by using scanning are essential.
Scan/MRM analysis mode supports the measurement of environmental pollutants by enabling the target analysis using MRM and exhaustive analysis using scan simultaneously. Here we introduce quantitative analysis results for trace amounts of PCBs and chlorinated pesticides using MRM data and show results of screening for environmental pollutants other than the target components for MRM measurement by applying the "GC/MS Database for Simultaneous Analysis for Environmental Analysis" to the scan data.

GCMS-TQ8040 Features

- Simultaneous Scan/MRM analysis using Advanced Scanning Speed Protocol (ASSP™)
- Equipped with a high-sensitivity ion source

GC/MS Database for Simultaneous Analysis for Environmental Analysis Features

- Approximate quantitative values of hazardous chemicals without standard samples can be confirmed.
- Includes 942 components for environmental pollutants

Experiment

1 mL of 1 mol/L phosphate buffer was added to 1 L of river water. 50 g of sodium chloride was then added. Liquid-liquid extraction was performed twice using 50 mL of dichloromethane, and the sample was concentrated to 1 mL after dehydration with anhydrous sodium sulfate. Afterwards, 100 µL of the 10 µg/mL internal standard solution was added to form the measurement sample.
The analysis conditions are shown in Table 1.

Table 1: Analysis Conditions

GC-MS	GCMS-TQ8040
Column	DB-5MS™ (Length 30 m, 0.25 mm I.D., df=0.25 µm) (Agilent J&W, P/N: 122-5532)
Glass Insert	Splitless Insert w/oWool (Shimadzu, P/N: 221-48876-05)
[GC]	
Injection Port Temp.	250 °C
Column Oven Temp.	40 °C (2 min.) → (8 °C /min.) → 310 °C (5 min.)
Injection Mode	Splitless
Carrier Gas Control	Linear velocity (40.0 cm/sec.)
Injection Volume	1 µL
[MS]	
Interface Temp.	300 °C
Ion Source Temp.	200 °C
Acquisition Mode	FAAST (Scan/MRM simultaneous measurement)
Scan Mass Range	*m/z* 45 to 600
Scan Event Time	0.15 sec.
Scan Speed	5,000 u/sec.

The river water sample was provided by Prof. Kadokami of the University of Kitakyushu.

Analysis Results

The MRM data results are shown in Fig. 1, and the scan data results are shown in Fig. 2 and in Table 2. The MRM analysis was able to quantify trace amounts of compounds with excellent sensitivity even at ppq to ppt level and analyze the target PCBs with high sensitivity and high resolution. Also, it was possible to identify and quantify 84 types of environmental pollutant from the scan data by using the Database for Simultaneous Analysis. Thus, performing simultaneous Scan/MRM measurements and scanning for unanticipated compounds enables the oversight of pollutants other than the target components to be avoided.

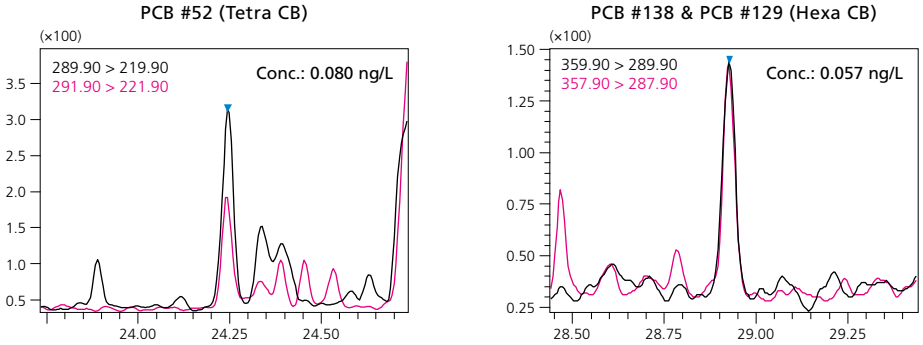


Fig. 1: Mass Chromatogram and Quantitative Results of the Target Components Using MRM Data

Table 2: Semi-Quantitative Results of Environmental Pollutants Detected Using Database Software for Simultaneous Analysis

Categ	Detected Compounds	Conc. (ng/L in water)	Categ	Detected Compounds	Conc. (ng/L in water)	Categ	Detected Compounds	Conc. (ng/L in water)
1	n-C11H24	62.7	1	Biphenyl	160.7	2	Coprostanol	14121.5
1	n-C12H26	120.1	1	Fluorene	44.6	2	Cyclohexanol	899.7
1	n-C13H28	241.3	1	Naphthalene	270.4	2	Ethanol, 2-phenoxy-	1989.0
1	n-C14H30	190.5	1	Phenanthrene	126.4	2	Phenylethyl alcohol	101.2
1	n-C15H32	188.6	2	Diphenyl ether	213.6	2	Stigmasterol	1697.2
1	n-C16H34	215.3	2	Isophorone	8601.1	2	1,3-Dichloro-2-propanol	304.5
1	n-C17H36	419.8	2	2-Methylphenol	506.7	3	2-Naphthylamine	93.4
1	n-C18H38	420.6	2	2-Naphthol	126.0	3	Acetamide, N-phenyl-	571.0
1	n-C19H40	435.1	2	2-Phenylphenol	52.4	3	2-Chloroaniline	533.5
1	n-C20H42	541.7	2	4-Methyl-2,6-di-t-butylphenol	167.6	3	3,4-Dichloroaniline	959.2
1	n-C21H44	677.3	2	4-tert-Octylphenol	155.4	3	Quinoline	175.4
1	n-C22H46	794.4	2	Bisphenol A	3169.6	4	2(3H)-Benzothiazolone	2544.3
1	n-C23H48	1118.2	2	Nonylphenol	9845.0	4	2-(Methylthio)-benzothiazol	277.7
1	n-C24H50	1505.7	2	2,4,5-Trichlorophenol	38.1	4	2-Acetyl-5-methylthiophene	65.4
1	n-C25H52	2383.4	2	2,4,6-Tribromophenol	3292.6	4	Benzothiazole	177.0
1	n-C26H54	1299.5	2	2,4,6-Trichlorophenol	44.7	5	Tris(1,3-dichloro-2-propyl) phosphate	677.0
1	n-C27H56	1392.3	2	Triclosan	211.3	6	Caffeine	1736.5
1	n-C28H58	966.2	2	Bis(2-ethylhexyl)phthalate	11036.3	6	Diethyltoluamide	345.0
1	n-C29H60	1445.2	2	Diethyl phthalate	2306.6	6	Ibuprofen	1596.3
1	n-C30H62	1625.2	2	Diisobutyl phthalate	914.1	6	L-Menthol	4281.0
1	n-C31H64	2393.8	2	Dimethyl phthalate	261.8	6	Thymol	223.1
1	n-C32H66	979.3	2	Di-n-butyl phthalate	807.1	6	Nicotine	846.2
1	n-C33H68	863.2	2	2-Butoxyethanol	4013.9	7	Fenobucarb	551.5
1	n-C9H20	152.0	2	2-Ethyl-1-hexanol	1628.3	7	Permethrin 1	661.7
1	4-Cymene	351.2	2	alpha-Terpineol	900.9	7	Permethrin 2	114.2
1	1,3-Dimethylnaphthalene	618.1	2	Benzyl alcohol	203.7	7	Piperonyl butoxide	85.7
1	2,6-Dimethylnaphthalene	403.9	2	beta-Sitosterol	3799.5	7	2-Phenylphenol (OPP)	63.9
1	2-Methylnaphthalene	100.9	2	Cholesterol	14408.1	7	Biphenyl	34.6

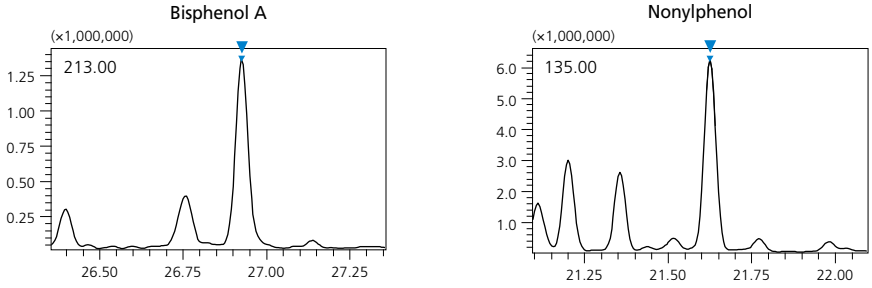


Fig. 2: Mass Chromatogram of Compounds Detected by Scan

Analysis of PBDEs in Sediment Using GC-MS/MS

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants (BFRs) widely used in plastics. Due to their toxicity and persistence, their industrial production is to be eliminated under the Stockholm Convention.
Contamination from PBDEs is widespread in the environment, including in sediments.
In order to determine PBDEs in sediments, they need to be separated from a large amount interferences co-extracted during sample preparation. GC-MS and GC-MS/MS were evaluated for this purpose.

GCMS-TQ8040 Features

- OFF-AXIS ion optics eliminates noise such as neutral ions.
- Equipped with a high-sensitivity ion source
- Cross-talk minimized by the high-efficiency collision cell UFsweeper™

Experiment

Method 1614 Native PAR Stock Solution and Method 1614 Labeled Surrogate Stock Solution (Cambridge Isotope Laboratories) were used as target compound and surrogate standards, respectively. Calibration solutions were prepared at concentrations of 10, 20, 50, 100, 500 ng/mL (10 times higher concentrations of deca-BDE). After the addition of surrogate, sediment samples were extracted using Soxhlet extractions. The extract solution was cleaned-up by sulfuric-acid treatment, removal of sulfur using copper, and Florisil treatments. SIM and MRM modes were used for GC-MS and GC-MS/MS, respectively. The analytical conditions are shown in Table 1.

Table 1: Analysis Conditions

GC-MS	GCMS-QP2010 Ultra
GC-MS/MS	GCMS-TQ8040
Column	Rtx™-1614 (Length 30m, 0.25mm I.D., df=0.1 µm) (Restek, P/N: 10295)
Glass Insert	Splitless Insert w/Wool (Shimadzu, P/N: 221-48876-03)
[GC]	
Injection Port Temp.	320 °C
Column Oven Temp.	140 °C (3 min.) → (5 °C /min.) → 320 °C (5min.)
Injection Mode	Splitless
Sampling Time	1 min.
Flow Control Mode	Linear velocity (47.9 cm/sec.)
High-Pressure Injection	150 kPa (1.2 min.)
Injection Volume	1 µL
[MS]	
Interface Temp.	300 °C
Ion Source Temp.	230 °C
GC-MS	
Acquisition Mode	SIM
SIM Event Time	0.5 sec.
Micro Scan Width	0.6 u
GC-MS/MS	
Acquisition Mode	MRM

Analysis Results

Fig 1. shows the MRM chromatogram of hepta-BDE (BDE-183) at 10 ng/mL. For eight PBDE congeners, calibration curves show linearity from 10 to 500 ng/mL (from 100 to 5000 ng/mL of deca-BDE) with a correlation coefficient of 0.9999 (Table 2).

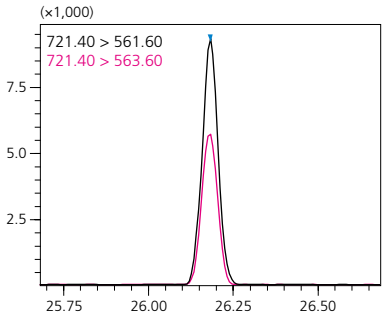


Fig. 1: MRM Chromatogram of Hepta-BDE (BDE-183)

Table 2: Correlation Coefficients of Calibration Curves for 8 PBDEs Congeners

Compound Name	R
Tri-BDE (BDE-028)	0.9999
Tetra-BDE (BDE-47)	0.9999
Penta-BDE (BDE-100)	0.9999
Penta-BDE (BDE-99)	0.9999
Hexa-BDE (BDE-154)	0.9999
Hexa-BDE (BDE-153)	0.9999
Hepta-BDE (BDE-183)	0.9999
Deca-BDE (BDE-209)	0.9999

Fig. 2 shows GC-MS SIM and GC-MS/MS MRM chromatograms. Several PBDEs could not be determined due to matrix interference in the SIM chromatogram, whereas those PBDEs were clearly detected in the MRM chromatogram. This demonstrates that the MRM mode allows the selective detection of target compounds even in the presence of large amounts of co-extracted interferences.

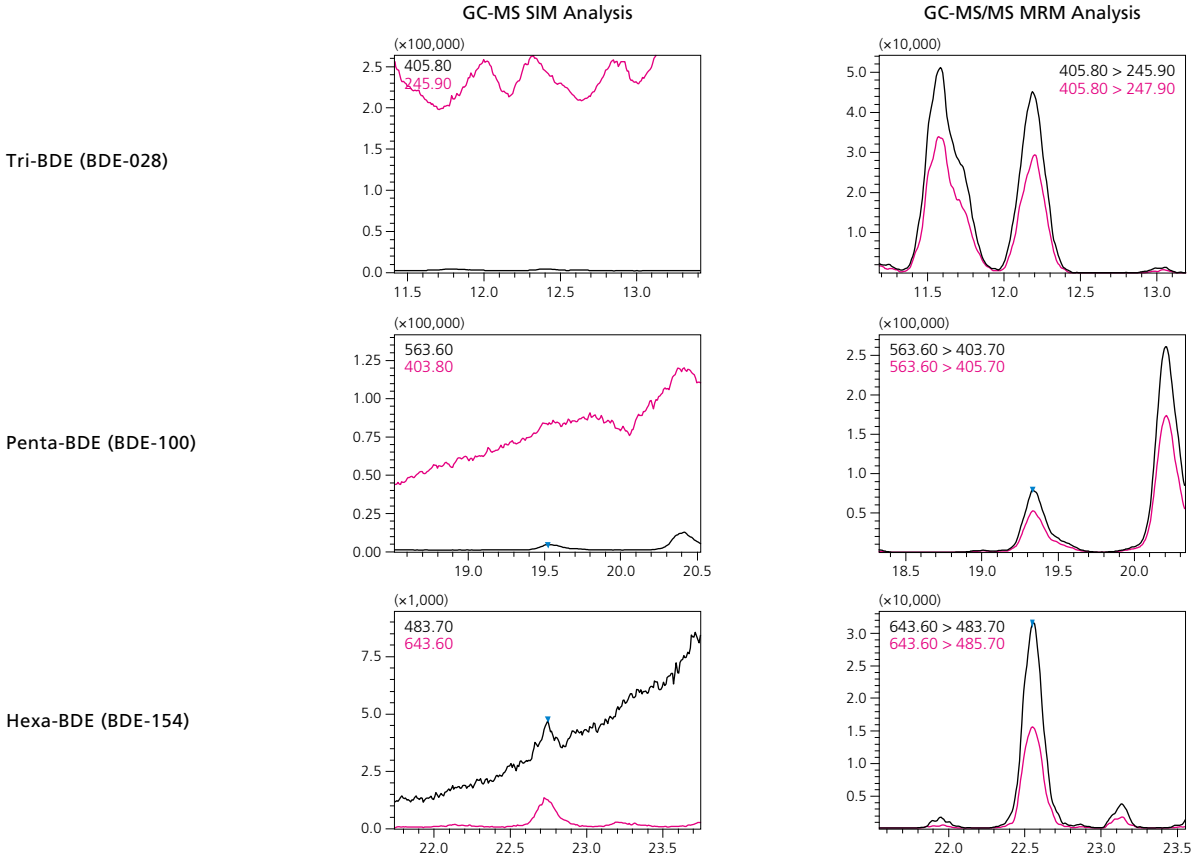


Fig. 2: SIM and MRM Chromatograms of Sediment Sample

Analysis of Metabolites in Rat Urine Using Scan/MRM

The GCMS-TQ8040 is a GC-MS/MS system equipped with Scan/MRM mode to allow simultaneous scan and MRM data measurements. Using Scan/MRM mode, it is possible to perform high-sensitivity analysis through high selectivity via MRM, while simultaneously performing qualitative analysis utilizing the mass spectrum via scan.
By making use of these features, the target components can be quantified reliably with MRM, and untargeted, unknown components can be qualified through a library search of the mass spectrum obtained with scan.
The results of Scan/MRM measurements of metabolites extracted from rat urine is reported here. Five target components are measured with MRM, and unknown peaks are identified through a library search of the mass spectrum obtained with scan.

GCMS-TQ8040 Features

- Simultaneous Scan/MRM analysis using Advanced Scanning Speed Protocol (ASSP™)
- Automatic creation of optimal methods by Smart MRM

Smart Metabolites Database Features

- Registers MRM information of 475 metabolites mainly contained in biological samples such as blood, urine and cells.
- Retention time correction using retention indices

Experiment

The urease-treated direct drying method[1] was used, and the rat urine was then subjected to trimethylsilylation prior to measurement. Scan/MRM was used as the measurement mode. Table 1 shows the analysis conditions.

Table 1: Analysis Conditions

GC-MS	GCMS-TQ8040
Column	DB-5™ (Length 30 m, 0.25 mm I.D., df=1.00 µm) (Agilent J&W, P/N: 122-5033)
Glass Insert	Splitless Insert w/Wool (Shimadzu, P/N: 221-48876-03)
[GC]	
Injection Port Temp.	280 °C
Column Oven Temp.	100 °C (4 min.) → (4 °C/min.) → 320 °C (0 min.)
Injection Mode	Splitless
Sampling Time	1 min.
Carrier Gas Control	Linear velocity (39.0 cm/sec.)
Injection Volume	1 µL
[MS]	
Interface Temp.	280 °C
Ion Source Temp.	200 °C
Acquisition Mode	Scan/MRM
Mass Range	*m/z* 45–600
Scan Event Time	0.2 sec.
Scan Speed	3,333 u/sec.

[1] I. Matsumoto, T. Kuhara, *Mass Spectrom. Rev.* 15 (1996) 43.

Analysis Results

Metabolites in rat urine were measured in Scan/MRM mode. The scan total ion chromatogram and the MRM mass chromatogram are shown in Fig. 1. Figs. 2 and 3 show the mass spectra measured with scan and the library mass spectra identified through the library search.

Using Scan/MRM mode, high-sensitivity analysis of target compounds and identification of unknown components can be obtained from a single data.

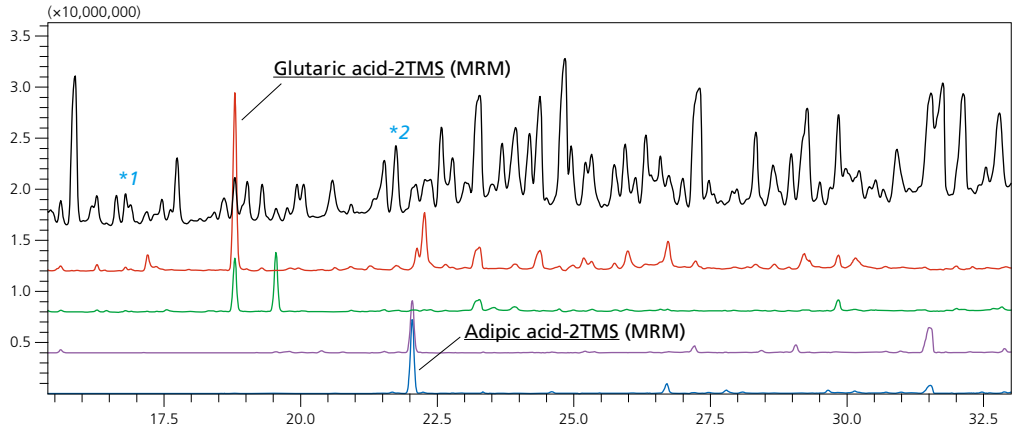


Fig. 1: Scan Total Ion Chromatogram from Scan/MRM Measurement of Metabolites in Rat Urine
Black: Scan Total Ion Chromatogram, Red: MRM Transition 158 > 116, Green: MRM Transition 158 > 101,
Purple: MRM Transition 275 > 141, Blue: MRM Transition 275 > 111

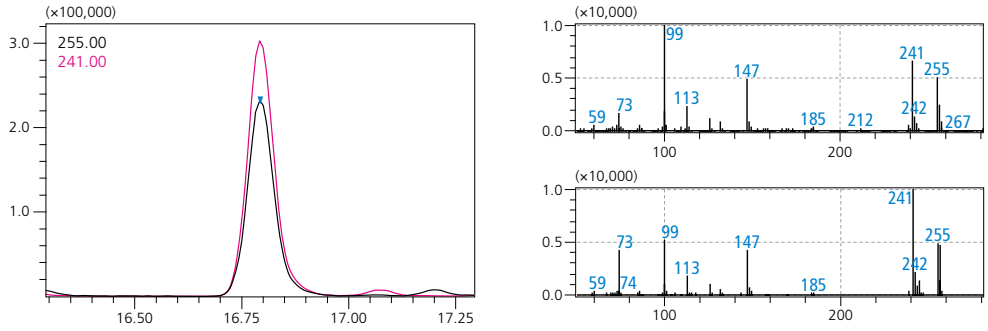


Fig. 2: Scan Mass Chromatogram and Mass Spectra for Uracil-2TMS (*1)
(Upper: Measurement spectrum, Lower: Library spectrum)

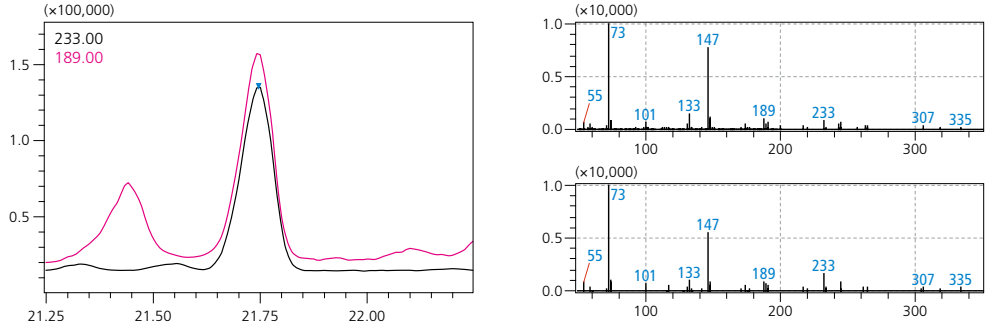


Fig. 3: Scan Mass Chromatogram and Mass Spectra for Malic Acid-3TMS (*2) (Upper: Measurement spectrum, Lower: Library spectrum)

Analysis of Metabolites in Serum Using GC-MS/MS

Single quadrupole GC-MS provides excellent chromatographic resolution and enables stable measurements, and is therefore widely utilized for metabolome analyses involving the comprehensive analysis of *in vivo* metabolites. However, biological samples contain many metabolites and various matrices, so separation with single quadrupole GC-MS can be difficult. With triple quadrupole GC-MS/MS MRM, MS separation is performed twice, with Q1 and Q3. This helps remove the impact of overlapping peaks due to interfering components in comparison with scan mode, in which MS separation is performed with a single quadrupole, and thus enables the acquisition of accurate quantitative results with high-sensitivity detection.
An analysis of metabolites in standard human serum using the scan and MRM methods included in the Smart Metabolites Database, as well as a comparison of the results is reported in this section.

GCMS-TQ8040 Features

- New firmware protocol enables MRM analysis with up to 32,768 transitions in a single analysis.
- Automatic creation of optimal methods by Smart MRM

Smart Metabolites Database Features

- Mainly contains metabolites included in biological samples, such as serum, urine, and cells
- Retention time correction using retention indices

Experiment

In the pretreatment process, 2-isopropylmalic acid was added as an internal standard to 50 µL of standard human serum, after which metabolites were extracted with a methanol/water/chloroform (2.5:1:1) solution. Methoxime and trimethylsilyl derivatives were then formed to obtain the samples[1]. The respective samples were measured six times each in scan and MRM modes using methods included in the Smart Metabolites Database. Table 1 shows the analysis conditions.

Table 1: Analysis Conditions

GC-MS	GCMS-TQ8040
Column	DB-5™ (Length 30 m; 0.25 mm I.D.; df = 1.00 µm) (Agilent J&W, P/N: 122-5033)
Glass Insert	Splitless Insert w/Wool (P/N: 221-48876-03)
[GC]	
Injection Port Temp.	280 °C
Column Oven Temp.	100 °C (4 min.) → (4 °C/min.) → 320 °C (8 min.)
Injection Mode	Splitless
Carrier Gas Control	Linear velocity (39.0 cm/sec.)
Injection Volume	1 µL
[MS]	
Interface Temp.	280 °C
Ion source Temp.	200 °C
Acquisition Mode	Scan
Mass Range	*m/z* 45–600
Event Time	0.3 sec.
Measurement Mode	MRM
Loop Time	0.3 sec.

[1] S. Nishiumi, M. Shinohara, A. Ikeda, T. Yoshie, N. Hatano, S. Kakuyama, S. Mizuno, T. Sanuki, H. Kutsumi, E. Fukusaki, T. Azuma, T. Takenawa, M. Yoshida, *Metabolomics* 6 (2010) 518–528

Analysis Results

Fig. 1 shows mass chromatograms for serum metabolites obtained in scan and MRM modes. In scan mode, some of the four components shown were not detected due to interfering components and insufficient sensitivity, resulting in a repeatability of 14 % or more. In contrast, favorable results were obtained with MRM, which eliminated the impact of interfering components, enabling high-sensitivity measurements with a repeatability of 6.5 % or less (see Table 2).

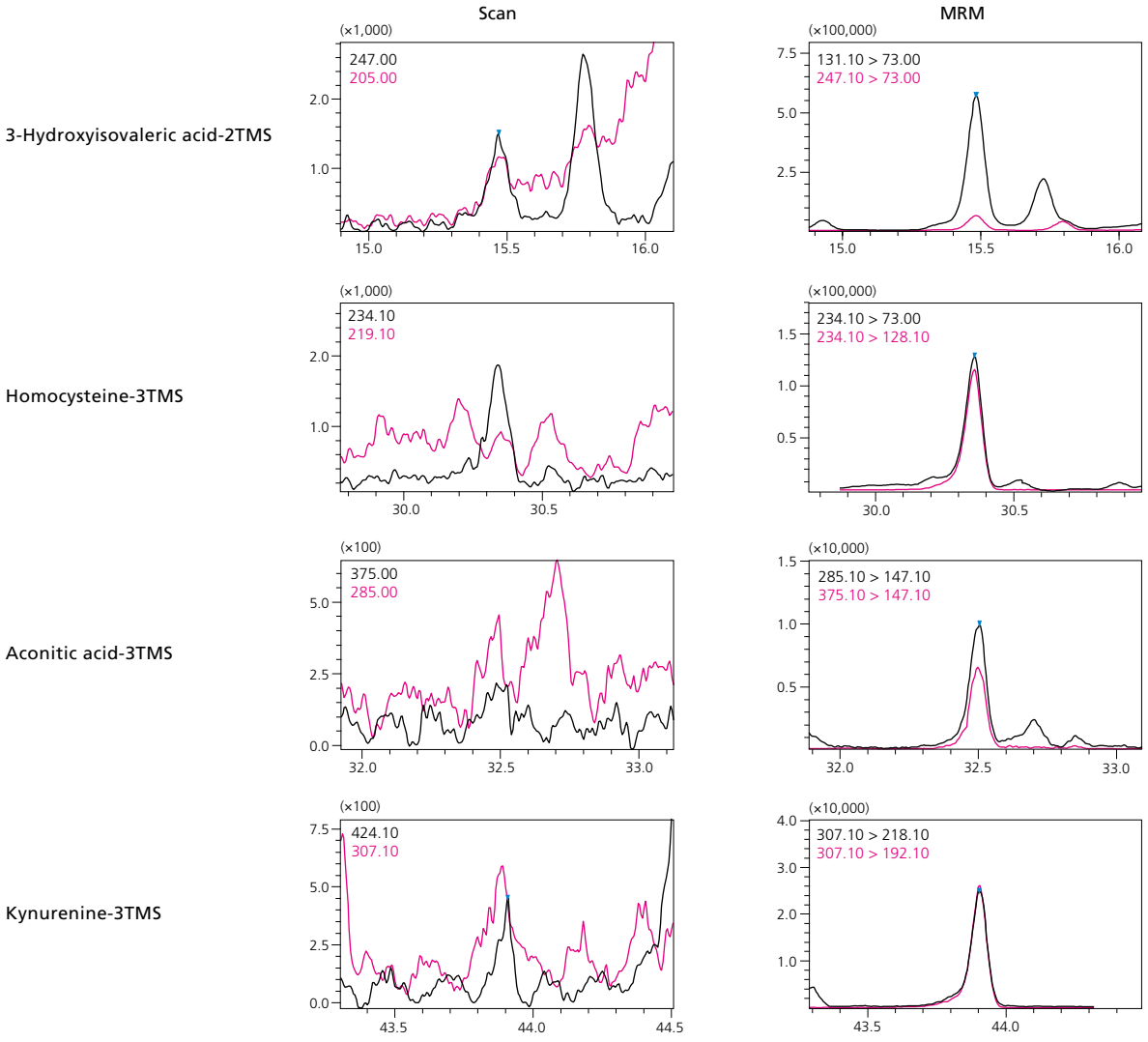


Fig. 1: Comparison of Scan (Left) and MRM (Right) Mass Chromatograms for Metabolites in Standard Serum

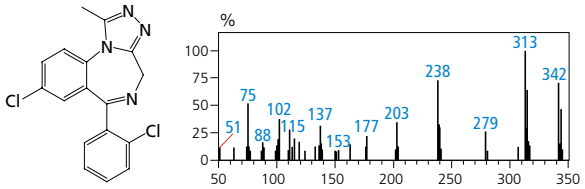
Table 2: Repeatability for Scan and MRM Modes

(corrected with internal standard)	%RSD (n=6)	
Compound name	MRM	Scan
3-Hydroxyisovaleric acid-2TMS	3.99	14.0
Homocysteine-3TMS	5.04	23.4
Aconitic acid-3TMS	5.98	N/A
Kynurenine-3TMS	6.48	24.5

Analysis of Psychotropic Drugs in Whole Blood Utilizing Simultaneous Scan/MRM Measurements

When analyzing medicinal toxicants using GC-MS, the presence of fatty acids and cholesterol, which exist in large quantities in whole blood, can interfere with detection. In fact, profiles for triazolam and etizolam, benzodiazepine psychotropic drugs, overlap with the cholesterol chromatogram, making data analysis difficult with a single GC-MS system. Furthermore, the retention indices for triazolam and etizolam are adjacent, and both have characteristic *m/z* ratios of 313 and 342, respectively (Fig. 1), making it even more difficult to distinguish these compounds.
Consequently, there are high expectations for utilizing GC-MS/MS. Here we introduce an example of simultaneous Scan/MRM measurements for the mass separation of cholesterol from triazolam and etizolam in whole blood and an example of simultaneous screening for other medicinal toxicants by applying scan data from simultaneous Scan/MRM measurements to the "GC/MS Forensic Toxicological Database".

Triazolam (retention index: 3134)



Etizolam (retention index: 3126)

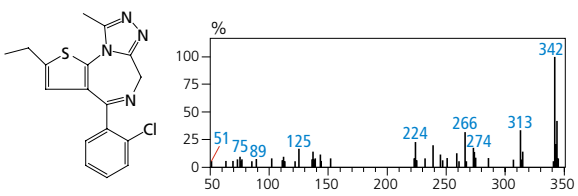


Fig. 1: Scan Mass Spectra and Retention Indices for Triazolam and Etizolam

GCMS-TQ8040 Features

- Simultaneous Scan/MRM analysis using Advanced Scanning Speed Protocol (ASSP™)
- OFF-AXIS ion optics eliminates noise such as neutral ions.

GC/MS Forensic Toxicological Database Features

- Contains a comprehensive range of forensic and toxicology-related compounds such as drugs of abuse, psychotropic drugs, and pesticides
- Retention time correction using retention indices

Experiment

The liquid-liquid extraction method via EXtrelut™ NT3 was used for pretreatment of the whole blood sample. For both the acidic fraction and basic fraction, 1 mL of the collected whole blood sample was measured, and each was then diluted with 1 mL of Milli-Q water. The acidic fraction was adjusted to a pH of 5 using 10 % perchloric acid, and the basic fraction was adjusted to a pH of 9 with 10% ammonia water. Each solution was poured into EXtrelut NT3, and after leaving to stand 30 minutes, eluted with a 10 mL mixed solution of chloroform and isopropanol (3 :1). Afterwards, the extracted acidic fraction and basic fraction liquids were mixed, and following desiccation with silica gel, dried and hardened under a nitrogen gas flow. The resulting sample was then re-dissolved with a 200 µL mixed solution of chloroform and isopropanol (3 :1).
In order to calculate semi-quantitative values utilizing the "GC/MS Forensic Toxicological Database", the custom internal standard (P/N: 560294, from Shimadzu GLC), which contains 8 PAH-d isomers, was adjusted to a concentration of 1 µg/mL for use as an internal standard sample. The adjusted extracted sample and the internal standard sample were injected simultaneously into the GC-MS/MS system using the AOC-20i+s solvent flush mode. The analytical conditions are shown in Table 1 (page 18).

Analysis Results

Figs. 2 and 3 show the mass chromatograms obtained from Scan/MRM measurements of the whole blood extracted sample (blank), which did not contain etizolam or triazolam, and the sample created by adding etizolam and triazolam to the blank sample in order to reach a concentration of 500 ng/mL. In the scan mass chromatogram, the cholesterol is detected at the same retention time as etizolam and triazolam, making it difficult to determine the presence of these psychotropic drugs. However, 2-stage mass separation via MRM enables separation from the cholesterol, making selective detection of etizolam and triazolam possible. In the scan measurement, similar mass spectra are indicated for etizolam and triazolam, but in the MRM chromatogram, they could be separated and confirmed without mutual influences.

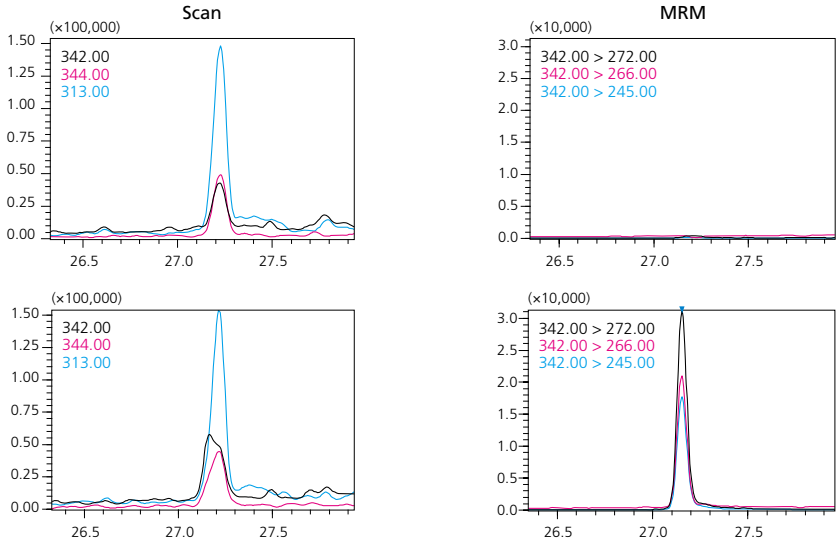


Fig. 2: Scan and MRM Mass Chromatograms of Etizolam in a Whole Blood Sample
(Left: Scan; Right: MRM; Top: Whole blood extracted sample (Blank); Bottom: Sampled created by adding triazolam and etizolam to the blank sample (500 ng/mL))

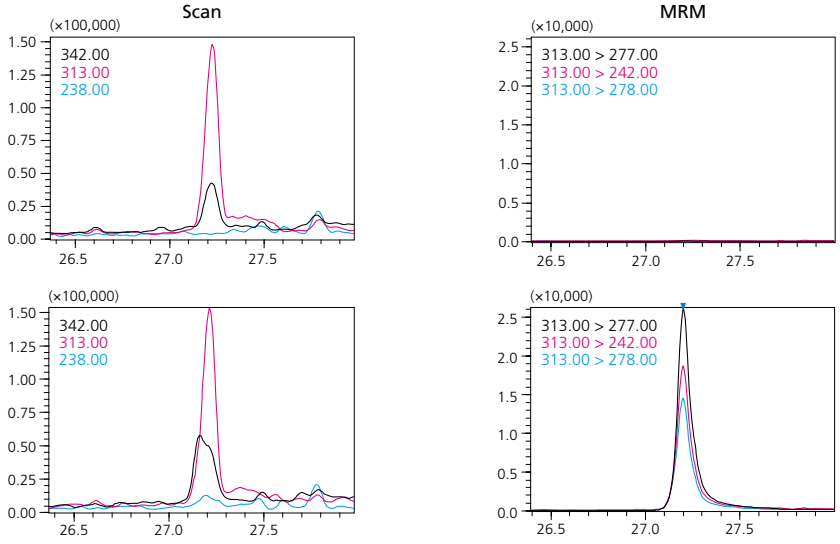


Fig. 3: Scan and MRM Mass Chromatograms of Triazolam in a Whole Blood Sample
(Left: Scan; Right: MRM; Top: Whole blood extracted sample (Blank); Bottom: Sampled created by adding triazolam and etizolam to the blank sample (500 ng/mL)

Table 1: Analysis Conditions

GC-MS	GCMS-TQ8040
Column	SH-Rxi™-5Sil MS (Length: 30 m; 0.25 mm I.D., df=0.25 µm) (Shimadzu, P/N: 221-75954-30)
Glass Liner	Splitless Insert w/Wool (P/N: 221-48876-03)

[GC]	
Injection Port Temp.	260 °C
Column Oven Temp.	60 °C (2 min.) → (10 °C/min.) → 320 °C (10 min.)
Injection Mode	Splitless
Flow Control Mode	Linear velocity (45.6 cm/sec.)
Injection Volume	1 µL

[MS]	
Interface Temp.	280 °C
Ion Source Temp.	200 °C
Acquisition Mode	Scan/MRM
Scan Event Time	0.15 sec.
Scan Mass Range	*m/z* 45–700
Scan Speed	5,000 u/sec.

MRM motoring *m/z*

Fig. 4 shows the scan chromatogram for the extracted whole blood sample, measured in the simultaneous Scan/MRM analysis. By applying the Forensic Toxicological Database to the scan data, it was possible to identify the benzodiazepine psychotropic drugs (diazepam and desmethyldiazepam). To date, the method utilized for compound identification has involved a library search of the peak mass spectrum detected with the total ion current chromatogram (TIC). However, the method utilizing the Forensic Toxicological Database uses the estimated retention times and characteristic *m/z* mass chromatogram (MC) for detection as shown in Fig. 5. Accordingly, it enables quick and easy determination of the presence or absence of low-concentration medicinal toxicants that cannot be confirmed with the conventional method. In addition, the mass spectra of the detected medicinal toxicants can be compared to registered standard mass spectra, thereby improving the reliability of the data analysis. By performing simultaneous Scan/MRM analysis, it becomes possible to simultaneously screen for components not targeted by MRM measurements.

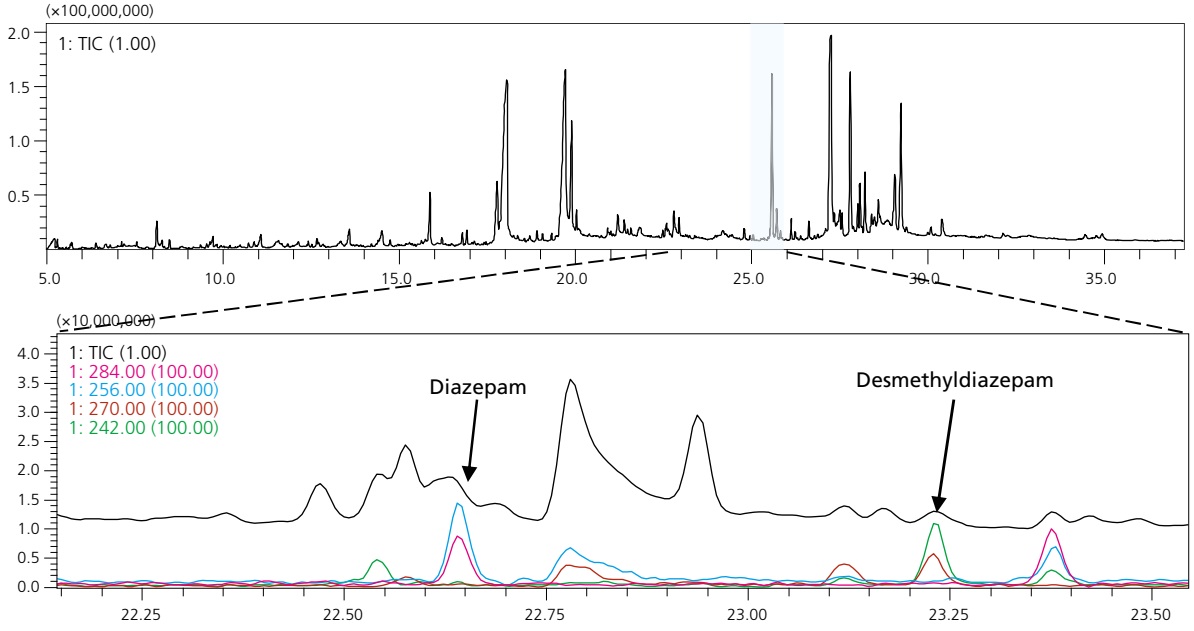


Fig. 4: Scan Chromatogram for Measured Whole Blood Sample
(Top: Scan TIC; Bottom: Enlarged TIC and MC for detected medicinal toxicants)

Comparison of Detected Peak Mass Spectrum and Standard Mass Spectrum

Data Analysis Utilizing Estimated Retention Times and Characteristic *m/z* Mass Chromatograms

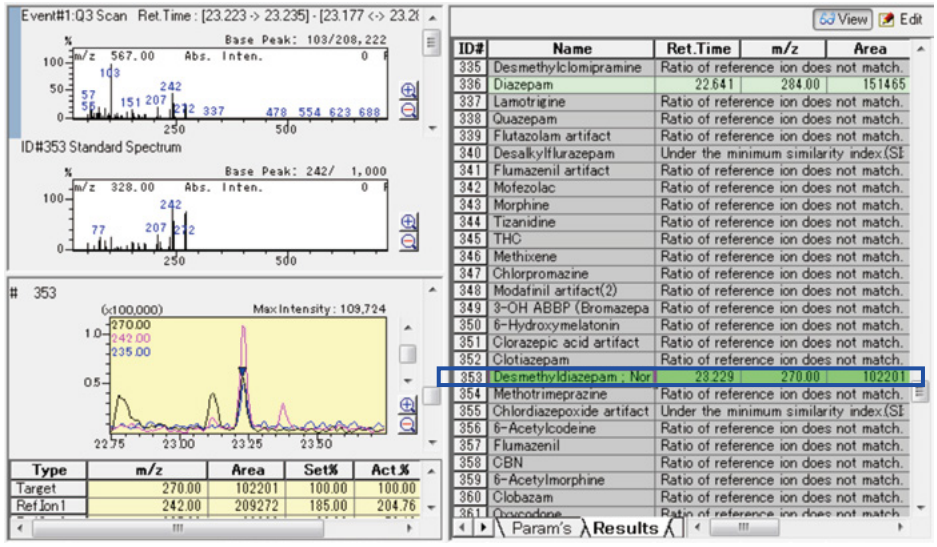


Fig. 5: Scan Data Analysis Window Using the "GC/MS Forensic Toxicological Database"

GC/MS Database

To begin a GC-MS/MS analysis, the analytical conditions and compound information for analysis must be set and these tasks require significant effort. Therefore, to facilitate the start of GC-MS/MS analyses, Shimadzu has preset the required information in various databases.

Pesticide Residue Analysis

GC/MS Residual Pesticides Database
- Quick-DB
- Smart Pesticides Database

Metabolic Component Analysis

Smart Metabolites Database

Environmental Pollutant Analysis

- Database for Simultaneous Analysis for Environmental Analysis
- Smart Environmental Database

Forensic Toxicological Analysis

- GC/MS Forensic Toxicological Database
- Smart Forensic Database

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