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Technical Report

Application for Plant Metabolome Analysis Using the GC/MS/MS Smart Metabolites Database

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Abstract:

The GC/MS/MS Smart Metabolites Database contains analytical conditions for the high-sensitivity detection of 475 metabolites commonly involved in the central metabolism of all biological organisms.
Using this database, 170 metabolites, including amino acids, organic acids, and sugars, were detected in mature tomato leaves. Furthermore, by verifying the analytical accuracy of an internal standard substance, ribitol, based on the relative elution time, detection intensity, and reference ion ratio, we confirmed that it is possible to quantitatively detect metabolites with a high level of accuracy.

Keywords: plants, metabolomics, extracts, GC/MS/MS

1. Introduction

The types and quantities of low-molecular-weight metabolites in plants determine the important characteristics of agricultural products such as color, flavor, and fragrance. Therefore, there is a demand for an efficient and broadly applicable metabolomic method specialized in analyzing plant metabolomes. In the present study, we performed a metabolomic analysis of mature tomato leaves using the GC/MS/MS Smart Metabolites Database, and then verified the analytical accuracy. This database enables users to perform a wide range of high-accuracy analyses after a short training period on the GC-MS/MS system.

2. Analytical Method

An internal standard substance, ribitol, was added to an 80% methanol extract of freeze-dried mature tomato leaves. The mixture was derivatized via methoximation and trimethylsilylation (TMS). To enable a comparison between the samples used in the analysis, a quality control (QC) sample was prepared for performing QC sample-based normalization by mixing equal amount of all samples. The QC sample was pretreated via a similar procedure. Subsequently, employing the conditions listed in Table 1 and using the MRM analytical method included in the Smart Metabolites Database, the samples were analyzed for 475 compounds.

Table 1 Analytical conditions

GC-MS	: GCMS-TQ8040
Column	: BPX-5 (30 m, 0.25 mm I.D., df=0.25 µm) (SGE, P/N : 054101)
Glass Insert	: Split insert with wool (RESTEK, P/N : 225-20803-01)
Autoinjector	: AOC-5000 Plus

GC	
Injection Port Temp.	: 250 °C
Column Oven Temp.	: 60 °C (2 min) → (15 °C /min) → 330 °C (3 min)
Injection Mode	: Split
Split Ratio	: 30
Carrier Gas Control	: Linear velocity (39.0 cm/sec)
Injection Volume	: 1 µL

MS	
Interface Temp.	: 280 °C
Ion Source Temp.	: 200 °C
Measurement Mode	: MRM
Loop Time	: 0.25 sec

3. Results

Fig. 1 shows an example of a total ion current (TIC) chromatogram obtained from the analytical measurements.

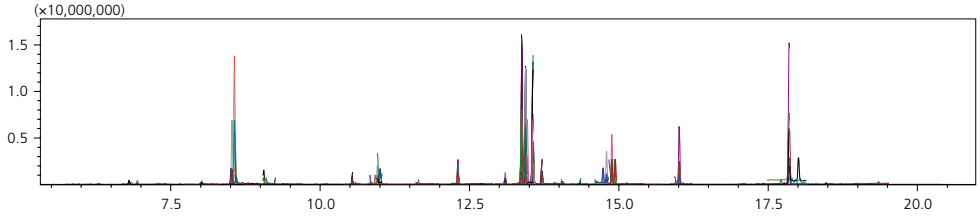

Fig. 1 TIC chromatogram of metabolite components present in mature tomato leaves

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The results confirmed that the internal standard substance ribitol can be detected with good repeatability. Fig. 2 shows a mass chromatogram of ribitol in the QC samples. Table 2 shows the relative elution time, detection intensity, and reference ion ratio in the QC samples and the corresponding variability (relative standard deviation, RSD).

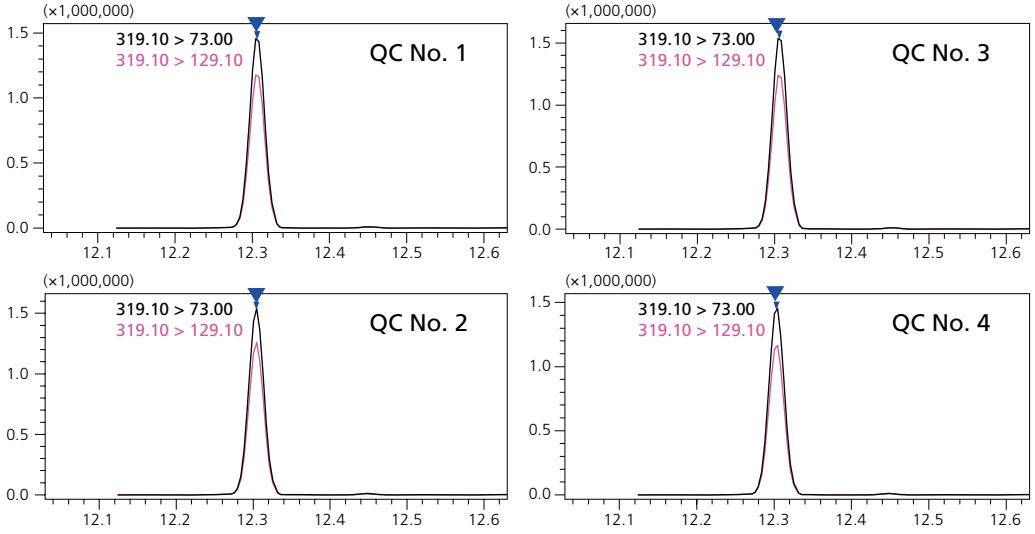

Fig. 2 Mass chromatogram of TMS-derivatized products of the internal standard

Table 2 Repeatability of ribitol detection

	Relative Elution Time (min.)	Detection Intensity (area value)	Reference Ion Ratio (%)
QC No. 1	12.306	2,097,224	80.44
QC No. 2	12.306	2,213,044	80.83
QC No. 3	12.304	2,187,242	81.50
QC No. 4	12.303	2,098,004	80.20
Average	12.305	2,148,879	80.70
%RSD	0.01	2.80	0.70

We detected 170 TMS-derivatized metabolites in the mature tomato leaves, as indicated in Table 3. Among the detected compounds, mass chromatograms of a sugar (glucose-meto-5TMS), organic acid (fumaric acid-2TMS), amino acid (valine-2TMS), and nucleoside (adenosine-4TMS) are shown in Fig. 3.

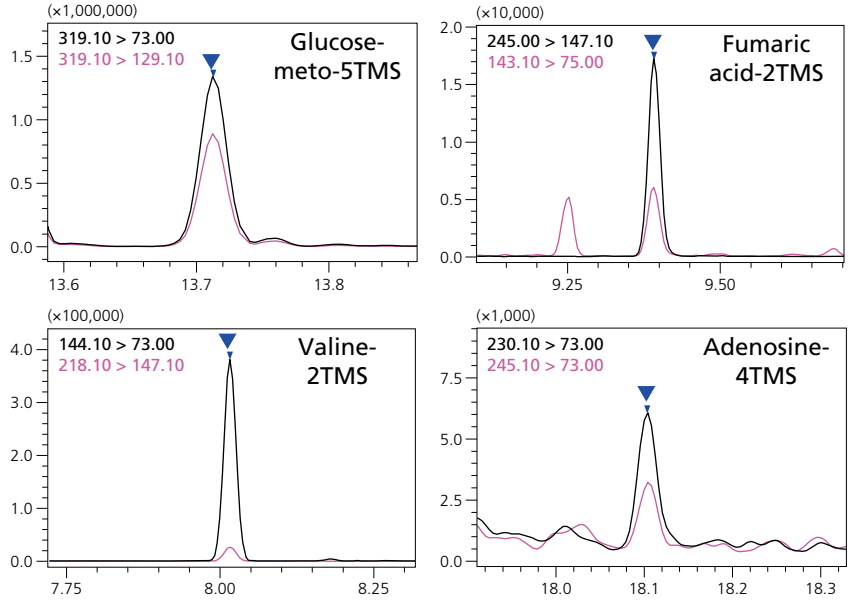

Fig. 3 Mass chromatograms of a sugar (glucose-meto-5TMS), organic acid (fumaric acid-2TMS), amino acid (valine-2TMS), and nucleoside (adenosine-4TMS)

Table 3 List of TMS-drivatized metabolites detected in mature tomato leaves by MRM analysis

1	1,6-Anhydroglucose-3TMS	61	Fumaric acid-2TMS	121	Norepinephrine-5TMS
2	2-Aminoadipic acid-3TMS	62	Galactosamine-5TMS(1)	122	Octopamine-4TMS
3	2-Aminoethanol-2TMS	63	Galactosamine-5TMS(2)	123	Oleamide-TMS
4	2-Aminoethanol-3TMS	64	Galactose-meto-5TMS(1)	124	Ornithine-4TMS
5	2-Aminopimelic acid-3TMS	65	Galactose-meto-5TMS(2)	125	Pantothenic acid-3TMS
6	2-Deoxy-glucose-4TMS(1)	66	Galacturonic acid-meto-5TMS(1)	126	Phenylalanine-2TMS
7	2-Deoxy-glucose-4TMS(2)	67	Galacturonic acid-meto-5TMS(2)	127	Phosphoric acid-3TMS
8	2-Deoxy-glucose-meto-4TMS	68	Glucaric acid-6TMS	128	Proline-2TMS
9	2-Hydroxyglutaric acid-3TMS	69	Gluconic acid-6TMS	129	Psicose-meto-5TMS(1)
10	2-Hydroxyhippuric acid-3TMS	70	Glucono-1,5-lactone-4TMS	130	Psicose-meto-5TMS(2)
11	2-Ketoadipic acid-meto-2TMS(2)	71	Glucosamine-5TMS(1)	131	Putrescine-4TMS
12	2-Ketoadipic acid-oxime-3TMS(2)	72	Glucosamine-5TMS(2)	132	Pyridoxal-meto-2TMS(1)
13	2-Ketoglutaric acid-meto-2TMS	73	Glucose 6-phosphate-meto-6TMS(1)	133	Pyridoxamine-3TMS
14	3-Aminoglutaric acid-2TMS	74	Glucose-meto-5TMS(1)	134	Pyruvic acid-meto-TMS
15	3-Aminopropanoic acid-3TMS	75	Glucose-meto-5TMS(2)	135	Rhamnose-meto-4TMS(1)
16	3-Dehydroshikimic acid-meto-3TMS(2)	76	Glucuronic acid-meto-5TMS(1)	136	Rhamnose-meto-4TMS(2)
17	4-Aminobutyric acid-2TMS	77	Glutamic acid 5-methylester-2TMS	137	Ribitol-5TMS
18	4-Aminobutyric acid-3TMS	78	Glutamic acid-3TMS	138	Ribonic acid-5TMS
19	4-Hydroxyphenyllactic acid-3TMS	79	Glutamine-4TMS	139	Ribose-meto-4TMS
20	4-Hydroxyphenylpyruvic acid-meto-2TMS	80	Glyceric acid-3TMS	140	Ribulose-meto-4TMS
21	5-Aminovaleric acid-3TMS	81	Glycerol-3TMS	141	S-Benzyl-Cysteine-4TMS
22	5-Dehydroquinic acid-meto-4TMS	82	Glycine-3TMS	142	Sedoheptulose 7-phosphate-meto-7TMS
23	5-Oxoproline-2TMS	83	Hippuric acid-TMS	143	Serine-2TMS
24	6-Phosphogluconic acid-7TMS	84	Histidine-3TMS	144	Serine-3TMS
25	Acetylglycine-2TMS	85	Histidinol-3TMS	145	Shikimic acid-4TMS
26	Aconitic acid-3TMS	86	Indol-3-acetic acid-2TMS	146	Sorbose-meto-5TMS(1)
27	Adenosine-4TMS	87	Inosine-4TMS	147	Sorbose-meto-5TMS(2)
28	Alanine-2TMS	88	Inositol-6TMS(2)	148	Spermine-6TMS
29	Allantoin-3TMS	89	Isocitric acid-4TMS	149	Suberylglycine-2TMS
30	Allantoin-4TMS	90	Isoleucine-2TMS	150	Sucrose-8TMS
31	Allose-meto-5TMS(1)	91	Isomaltose-meto-8TMS(1)	151	Tagatose-meto-5TMS(2)
32	Allose-meto-5TMS(2)	92	Isomaltose-meto-8TMS(2)	152	Tartaric acid-4TMS
33	Arabinose-meto-4TMS	93	Kynurenine-2TMS	153	Threitol-4TMS
34	Arabitol-5TMS	94	Kynurenine-3TMS	154	Threonic acid-4TMS
35	Arginine-3TMS	95	Lactitol-9TMS	155	Threonine-3TMS
36	Ascorbic acid-4TMS	96	Lactose-meto-8TMS(1)	156	Trehalose-8TMS
37	Asparagine-4TMS	97	Lactose-meto-8TMS(2)	157	Triethanolamine-3TMS
38	Aspartic acid-3TMS	98	Leucine-2TMS	158	Tryptamine-2TMS
39	Batyl alcohol-2TMS	99	Lysine-4TMS	159	Tryptophan-3TMS
40	Biotin-3TMS	100	Lyxose-meto-4TMS(1)	160	Tyramine-3TMS
41	Cadaverine-4TMS	101	Lyxose-meto-4TMS(2)	161	Tyrosine-3TMS
42	Citramalic acid-3TMS	102	Malic acid-3TMS	162	Urea-2TMS
43	Citric acid-4TMS	103	Maltitol-9TMS	163	Ureidopropionic acid-3TMS
44	Cytidine-4TMS	104	Maltose-meto-8TMS(1)	164	Ureidosuccinic acid-3TMS
45	Dihydroorotic acid-3TMS	105	Maltose-meto-8TMS(2)	165	Uridine-3TMS
46	Dihydrouracil-TMS	106	Mannitol-6TMS	166	Urocanic acid-2TMS
47	Dihydroxyacetone phosphate-meto-3TMS(1)	107	Mannose 6-phosphate-meto-6TMS(1)	167	Valine-2TMS
48	Dihydroxyacetone phosphate-meto-3TMS(2)	108	Mannose 6-phosphate-meto-6TMS(2)	168	Xylose-meto-4TMS(1)
49	Docosahexaenoic acid-TMS	109	Mannose-meto-5TMS(1)	169	Xylose-meto-4TMS(2)
50	Docosapentaenoic acid-TMS	110	Mannose-meto-5TMS(2)	170	Xylulose-meto-4TMS
51	Dopa-4TMS	111	Melatonin-TMS		
52	Dopamine-4TMS	112	meso-Erythritol-4TMS		
53	Erythrose 4-phosphate-meto-4TMS(1)	113	Methoprene acid-TMS		
54	Erythrose 4-phosphate-meto-4TMS(2)	114	Metoprolol-2TMS		
55	Erythrulose-meto-3TMS(1)	115	N-Acetyl-Lysine-2TMS		
56	Erythrulose-meto-3TMS(2)	116	N-Acetylmannosamine-meto-4TMS(1)		
57	Fructose 6-phosphate-meto-6TMS	117	N-Acetylmannosamine-meto-4TMS(2)		
58	Fructose-meto-5TMS(1)	118	N-Acetylneuraminic acid-6TMS		
59	Fructose-meto-5TMS(2)	119	N-Acetyl-Ornithine-4TMS		
60	Fucose-meto-4TMS(1)	120	N-Acetyltyrosine-2TMS		

4. Summary

A wide range of metabolites (475 components) can be analyzed using the GC/MS/MS Smart Metabolites Database. We detected 170 TMS-derivatized metabolites in mature tomato leaves, including multiple substance groups involved in the central metabolism of mature tomato leaves, such as sugars, amino acids, and organic acids. RSD was < 5 % for all internal standard substance detection accuracy criteria (relative elution time, detection intensity, and reference ion ratio), indicating that quantitative comparisons of metabolites in plant samples are possible. Using this application for plant metabolome analysis, metabolic products corresponding to a wide range of agricultural attributes can be examined.

Acknowledgments

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Triple Quadrupole Gas Chromatograph Mass Spectrometer

GCMS-TQ8040

Smart Performance That Boosts Routine Analytical Work

GC-MS/MS is useful for measuring trace quantities of various chemical substances present in a variety of sample types. However, specifying several parameter settings and employing suitable methods are required when using this technique.
Nevertheless, GCMS-TQ8040 can dramatically increase the productivity by automating tedious method creation processes and simultaneously analyzing multiple components with high sensitivity.

Smart Productivity

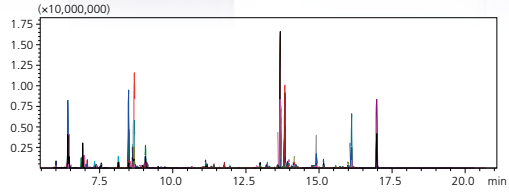
- Includes a new firmware protocol.
- Simultaneously analyzes a wide range of compounds with high sensitivity and high accuracy.
- Twin Line MS system minimizes the replacement of columns.

Smart Operation

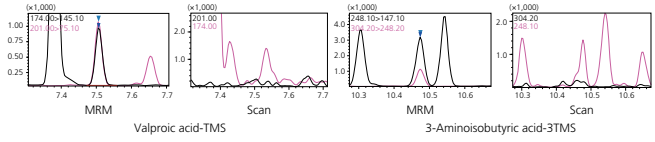
- Smart MRM automatically creates optimized methods.
- Automatically searches for optimal transitions.
- AART function automatically adjusts retention times.

Smart Performance

- Patented high-sensitivity ion source technology offers even higher sensitivity.
- OFF-AXIS Ion Optics reduces noise.
- Capable of performing high-sensitivity analysis even as a single GC-MS system.



TIC chromatogram obtained via the MRM analysis of metabolic components in standard human plasma.



Comparison of mass chromatograms of metabolites present in standard human plasma

Brochure: C146-E251

Database for GC/MS Analysis of Metabolites

Smart Metabolites Database

Simultaneous MRM Measurement of 475 Components Enabling the Detection of Trace Components

The GC/MS and GC/MS/MS database software supports metabolite measurements.
Measurements using the GCMS-TQ system enable high-sensitivity and separation MRM analysis. Metabolomics measures samples comprising a large number of metabolites and a wide variety of matrices. Therefore, it is difficult to analyze many compounds because of overlapping component peaks.
However, such effects can be avoided by using MRM measurements and performing mass separation twice. Consequently, MRM can detect components that were previously not detectable using conventional scan and SIM methods.

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