Application Note 10419

Metabolomics of Rice Genotypes using GC-MS/MS

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Introduction

Metabolomics is a high-throughput platform used to profile the small molecules or metabolites involved in metabolic processes. As a result of myriad biochemical reactions, metabolites are in a constant state of change. A metabolome signature, therefore, reflects the physiological status of a given sample in a living organism[1].

In plants, metabolome changes occur during development due to genetic influences and external stimuli such as environmental stressors. As such, the metabolome signature is a snapshot of the biological state of an organism at a specific time. The identification and quantification of these metabolites may then be used to 'fingerprint' or map the status of metabolome. It is estimated that more than 200,000 metabolites exist in the plant kingdom, encompassing a diverse range of compound structures[2].

Metabolome analysis poses major technical challenges due to the diversity and dynamic range of metabolites. In order to identify and quantify a whole range of metabolites, it is necessary to use several different analytical platforms.

Gas chromatography coupled with mass spectrometry (GC-MS) is particularly well suited to the study of low molecular-weight metabolites that can be made amenable to gas chromatography by chemical derivatization. However, while metabolite profiling by GC-MS in full scan mode reports identities of metabolites and their normalized relative intensities, the ultimate requirement for a quantitative description of the metabolite pool is absolute concentration determination. When studying a plant such as rice, targeted metabolites need to be accurately quantified for use as tools for functional genomics or as biomarkers to discriminate between different traits of diverse rice quality.

A triple-quadrupole mass spectrometer is a valuable tool as a detector in metabolomic studies because it enables identification and quantification of analytes in a complex organic samples[3,4]. The main advantage of GC-MS lies in its capability for both metabolite profiling and targeted metabolite quantitation for the analysis of hundreds of metabolites in one GC-MS/MS multiple reaction monitoring (MRM) run. The MRM acquisition mode of unique and timed precursor ion-product ion transitions for metabolites of interest eliminates interference from matrix or noise. Likewise, co-eluting compounds can be quantified separately, forgoing the need for deconvolution by mathematical methods.

As a food source, rice is a vital resource for our planet, responsible for roughly 21% of human caloric intake (http://www.knowledgebank.irri.org/). The metabolomic study of rice may be used to 'fingerprint' different rice samples in order to identify strains that hold significant promise for development, such as rice quality improvement and increased nutritional content. The International Rice Research Institute (IRRI), based in Manila, Philippines, is the largest non-profit agricultural research center in Asia. IRRI actively conducts research aimed at developing new rice varieties, which boost yield and maximize nutritional content. In this case study, we aim to test the suitability of the GC-MS/MS platform

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to profile the primary metabolism targets from mature rice grain of genetically diverse lines. The work reported here represents a collaboration with IRRI, aimed at establishing a procedure for metabolomic profiling of rice species.

Sample Preparation

Derivatized rice extracts were prepared by applying the optimized method described by Zhou et al.[5] for metabolic profiling of brown rice samples. Freshly dehulled grains were immediately dipped in liquid nitrogen prior to grinding the rice samples using a 60 mesh sieve. 300 mg of the ground brown rice sample was weighed, 3 mL of methanol/water (4 : 1 v/v) added as well as 100 µL capric acid solution (0.30 mg/mL) as internal standard. The sample vial was vortexed for 1 minute and let stand for 30 minutes followed by 60 minutes of sonication before centrifuging at 12,000 g for 10 minutes. 2 mL of the supernatant was removed and freeze-dried and subsequently derivatized using 90 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) and 80 µL of pyridine. The mixture was heated in a water bath at 75 ℃ for 45 minutes before being transferred to 2 mL GC vials with 200 µL inserts, ready for analysis by GC-MS/MS. Steps were followed to ensure that the derivatized samples were analyzed within 24 hours after derivatization[5].

Experimental Conditions

Twelve derivatized rice extracts were analyzed using the Thermo Scientific™ TSQ™ 8000 triple quadrupole GC-MS/MS system, equipped with the Thermo Scientific™ TRACE™1310 GC with an SSL Instant Connect SSL module and Thermo Scientific™ TriPlus RSH™ autosampler. Method details are provided in Table 1.

Sample Measurements

The metabolomics workflow developed for this study involved two distinct phases, implemented on the same TSQ 8000 mass spectrometer unit, as illustrated in Figure 1.

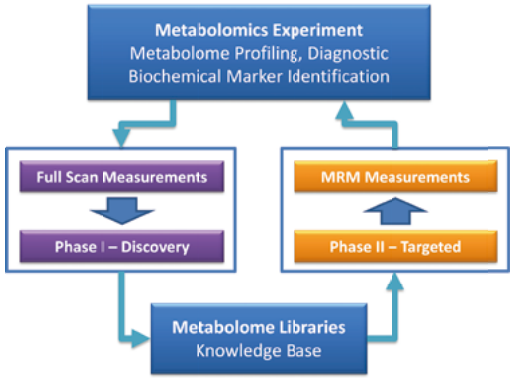

Figure 1. Metabolomics workflow showing the use of full scan and MRM measurements for discovery and targeted analyses respectively.

Table 1. GC conditions

Column type	Thermo Scientific™ TRACE™ TR-5MS
Column dimensions	length 30 m, ID 0.25 mm, film thickness 0.25 µm (P/N 260F142P)
Injector, temperature	Instant Connect SSL at 285 °C
Split flow	12 mL/min
Injection mode, volume	Split 1:10
Carrier gas, flow	helium, constant flow 1.2 mL/min
Oven program	60 °C, 4 min 8 °C/min to 170 °C 4 °C/min to 300 °C 300°C, 15 min
Transfer line temperature	285 °C

Table 2. Phase 1, Discovery MS Conditions—Full Scan

Ionization	EI, 70 eV
Scan mode, range	Full scan, 40–600 Da
Acquisition rate	250 ms
Ion source temperature	300 °C

Table 3. Phase 2, Targeted MS Conditions—SRM

Ionization	EI, 70 eV
Scan mode, range	Simultaneous SRM/Full scan Full scan, 40–600 Da SRM mode, see Appendix 1 below for SRM transitions
Acquisition rate	10 ms
Ion source temperature	300 °C

Figure 1 shows the general metabolomics workflow, with the progression from Phase I involving the discovery of compounds present as untargeted profiling, followed by Phase II, which involves the quantitation of the compounds identified in Phase I as a targeted approach.

Phase I requires full scan GC-MS analysis to allow for identification of compounds by searching library databases.

Phase II requires analysis in the selected reaction monitoring mode (SRM) in order to accurately quantitate compounds at trace levels in the heavy matrix background.

The multitude of individual SRM transitions for all potential marker compounds comprise the final multiple reaction monitoring method (MRM) applied for selective quantitation.

The TSQ 8000 mass spectrometer has the advanced capability of performing both full scan and SRM

measurements simultaneously, enabling the direct correlation of data collected in Phases I and II. The ability to perform simultaneous full scan and SRM analyses in a single sample run allows the users to quantitate the known metabolites (targeted), while allowing identification of unknowns (discovery). The compound retention time is constant for both workflow stages, serves as additional qualifier for identification, and greatly facilitates the setup of the targeted MRM method. The Thermo Scientific TSQ 8000 triple quadrupole GC-MS/MS system is hence ideally suited to the metabolomics workflow for both profiling and quantification of wide range of target compounds.

The application of TSQ 8000 GC-MS/MS system was then used for Phase I and II of this metabolomic study to understand grain quality preferences.

Results

Phase I – Discovery

During phase I of the workflow, five different rice samples were analyzed. These samples were chosen for their diverse characteristics and the likelihood they would display significant differences in the amounts of various metabolites. The full scan chromatograms of the tested samples are shown in Figure 2.

1. Precursor ion study—From full scan data, 66 metabolites of interest were selected and 2–3 precursor ions were chosen for each compound. This step required a single sample injection GC-MS/MS run and the selected precursor ions were then taken to the product ion study.
2. Product ion study—Product ions were selected for each of the precursor ions identified in step 1, above. Several product ions were chosen for each compound, resulting 3–7 precursor-product ion pairs per compound, which were then taken for the SRM optimization study.
3. SRM optimization—The collision energy for each precursor-product ion pair was optimized to give the maximum ion intensity and hence, sensitivity. Ion intensity is increased by quickly stepping the collision energy during the elution of the compounds of interest. The ability to directly control the instrument in real time allows many collision energy values to be measured in a single run, thereby minimizing greatly the time required for SRM method development and optimization. The SRMs for the 66 compounds were then optimized in only 5 sample injections.

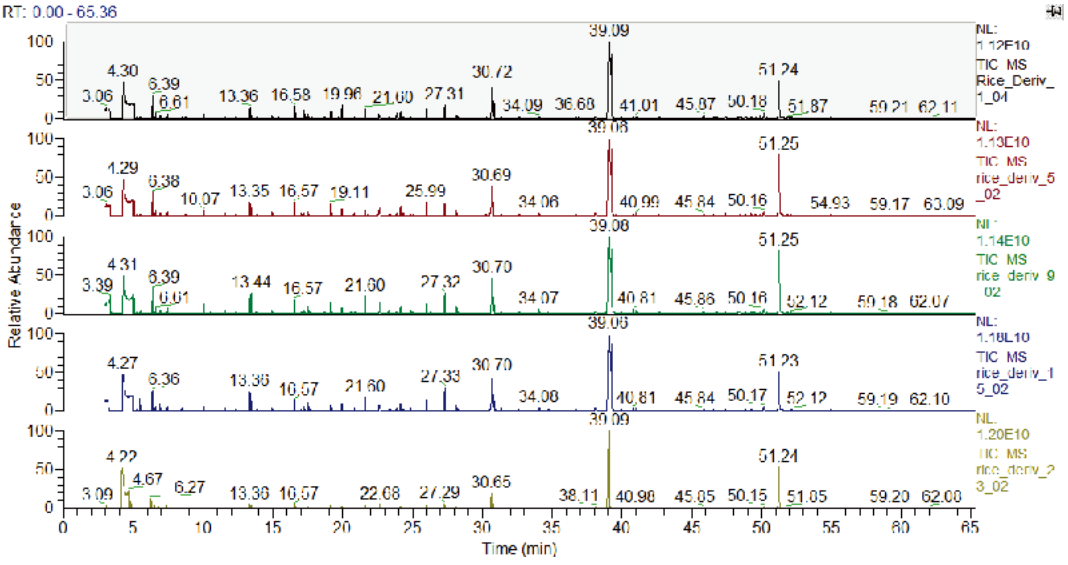

Figure 2. Full scan chromatograms of the five diverse rice samples

One of these samples (sample 3) was chosen for development and optimization of a complete SRM method. It had a large number of potentially interesting metabolites present.

The TSQ 8000 triple quadrupole mass spectrometer includes AutoSRM, a software package enabling fast and automated development and optimization of an SRM analysis method. This software was used to create an optimized SRM method using the following steps:

Deconvolution of the complex chromatograms was performed using the Automated Mass Spectral Deconvolution and Identification Software (AMDIS) provided with the TSQ 8000 instrument, together with the U.S. National Institute of Standards and Technology (NIST) mass spectral library and search software5. The 66 compounds selected in the AutoSRM method development described above were identified by library matching the collected full-scan spectra to those in the 2011 NIST mass spectral library.

Several of the mono- and di-saccharides were isomeric with identical mass spectra, so the library returned identical compounds for some of these peaks. However, these compounds could be resolved with the use of suitable reference standards and retention index comparison. The compounds identified included amino acids, organic and fatty acids, alcohols, and sugars (mono-, di-saccharides). The compounds detected and their corresponding SRM transitions are given in Appendix 1.

Phase II – Targeted Quantitation

The SRM transitions from the AutoSRM optimization described above were exported to the TraceFinder quantitation software and a processing method was automatically created. Rice sample 5 was then run again in SRM mode to check the validity of the SRM optimization, as shown in Figure 3.

Repeatability of the metabolite analysis method was determined by performing 10 repeat injections of derivatized rice extract 9 and calculating the RSD for each of the 66 compounds. Repeatability is illustrated in Figure 4.

The metabolites investigated produced highly reproducible peak areas, considering the heavy matrices of the rice extracts and the very low concentrations of many of the compounds. The average RSD for the 66 compounds was 7.8% and the RSD was less than 15% for 96% of the metabolites studied and less than 10% for 74% of the analytes.

The optimized MRM method was used to analyze 12 different rice strains to identify potential biomarkers, compounds that could be used to discriminate between the different rice accessions. The relative amounts of the individual metabolites can be represented in the form of a 'heat map', as shown in Figure 5.

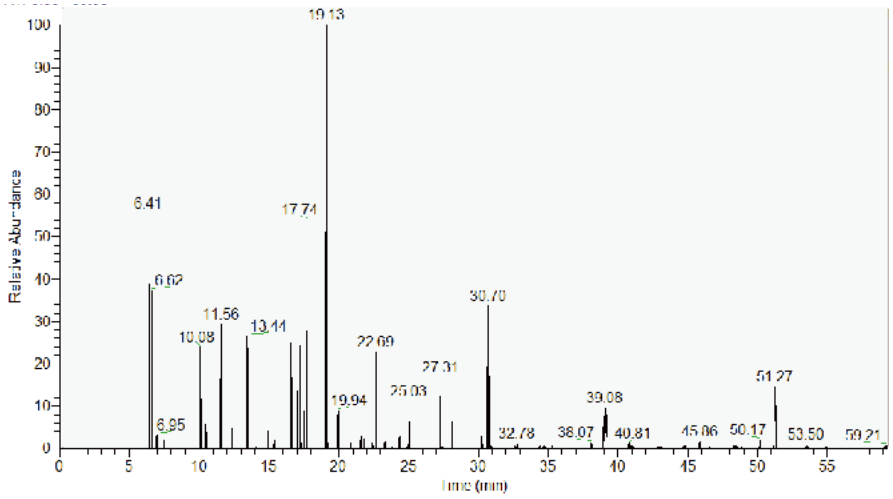

Figure 3. SRM chromatogram for rice sample 5.

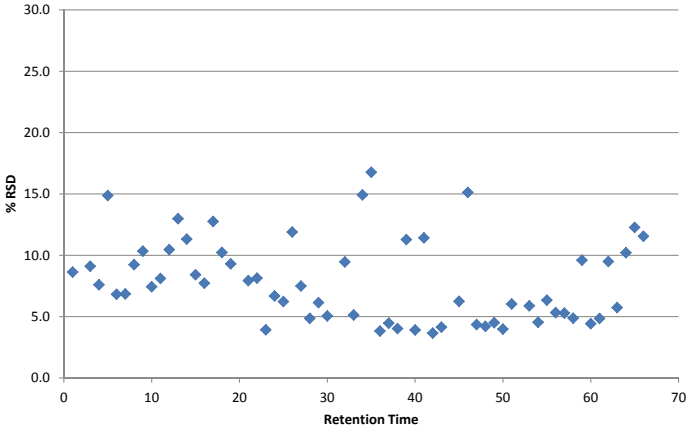

Figure 4. Peak area repeatability for the 66 compounds analyzed in rice sample 9.

Compound Number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
1	14	41	39	67	100	69	53	54	21	51	79	76
2	18	16	100	17	77	21	19	45	39	20	17	41
3	58	26	20	25	45	7	22	3	100	33	20	54
4	76	41	60	37	41	28	70	9	100	71	35	64
5	98	41	31	30	30	7	56	8	100	51	32	56
6	38	44	62	38	62	99	49	100	95	32	90	37
7	69	56	74	96	70	99	81	66	85	100	72	100
8	44	40	97	44	33	56	51	50	100	44	41	57
9	100	64	73	49	29	19	51	7	68	24	20	66
10	100	17	21	14	30	45	16	65	34	16	14	31
11	66	43	67	45	69	100	66	48	93	70	70	66
12	28	20	49	23	39	39	26	100	30	18	16	26
13	30	35	27	30	42	30	83	37	100	74	32	42
14	100	44	5	51	9	79	30	33	32	59	48	12
15	29	26	31	32	37	33	41	100	36	42	37	34
16	14	19	17	16	11	11	16	29	24	33	100	9
17	6	3	11	2	3	6	5	18	10	1	100	3
18	23	3	6	1	3	100	2	2	2	1	3	10
19	18	56	74	38	31	32	42	50	12	40	100	38
20	22	46	83	48	63	33	42	80	27	31	100	45
21	36	18	23	66	97	58	43	99	98	100	16	50
22	100	100	100	100	100	100	100	100	100	100	100	100
23	53	91	24	31	15	4	35	2	100	28	41	80
24	78	15	20	17	4	100	8	37	43	14	5	10
25	64	100	55	30	52	70	40	0	69	30	0	0
26	29	37	41	25	46	27	36	100	29	36	22	51
27	33	20	16	37	24	25	11	24	64	9	100	15
28	23	6	16	7	12	29	15	100	28	21	7	8
29	19	12	100	14	77	20	15	35	31	17	12	37
30	57	100	79	87	74	75	73	62	59	43	26	68
31	6	9	13	22	27	31	23	38	67	18	100	12
32	20	15	17	64	27	59	21	37	100	89	76	13
33	16	9	28	11	20	43	22	100	30	24	14	15
34	18	18	25	38	42	17	15	36	100	32	56	6
35	77	46	29	69	37	22	23	65	35	100	36	24
36	38	72	27	38	47	82	50	100	9	32	20	61
37	53	36	33	100	6	18	10	24	6	16	9	8
38	72	86	53	75	60	100	79	77	83	76	95	71
39	22	36	49	13	15	18	26	68	16	10	100	23
40	33	40	39	21	64	79	22	100	53	34	56	39
41	68	72	92	40	46	66	45	27	100	35	23	36
42	33	54	61	46	55	44	31	59	100	55	45	90
43	17	15	100	15	76	20	19	0	37	15	16	39
44	50	54	61	40	53	66	38	67	95	39	100	42
45	39	54	38	31	40	57	39	37	48	45	100	40
46	27	16	30	14	21	41	29	100	47	21	15	15
47	56	43	32	38	80	70	49	93	100	78	26	50
48	39	38	34	40	32	100	30	57	54	21	87	32
49	86	100	81	15	74	75	68	96	52	21	74	53
50	16	10	47	11	27	17	17	100	26	29	18	24
51	29	26	100	28	42	38	22	62	39	28	22	41
52	53	52	88	40	100	64	47	0	75	60	34	64
53	26	21	34	26	65	68	21	100	62	32	24	38
54	19	4	11	34	5	12	11	11	44	4	100	8
55	54	35	49	26	19	40	18	100	58	56	12	17
56	13	40	10	81	54	95	100	29	14	49	32	9
57	24	26	95	50	74	100	22	53	20	51	43	72
58	26	52	36	79	38	92	30	43	30	23	42	100
59	7	66	12	6	5	18	100	28	61	10	66	8
60	58	70	52	37	100	84	91	94	66	52	100	47
61	10	6	11	5	25	43	13	100	13	6	76	7
62	28	32	22	17	52	45	25	100	41	27	23	28
63	27	27	43	21	13	94	37	100	33	20	17	20
64	26	22	18	16	11	100	20	91	25	17	13	14
65	53	30	27	29	28	55	51	39	100	31	32	51
66	48	26	100	38	53	42	34	82	56	35	25	41

Figure 5. Rice extract samples 1–12. SRM comparison heat map for 66 compounds (compound numbers refer to numbering of compounds as in Appendix 1).

The heat map in Figure 5 shows the relative concentration of the 66 analytes in the 12 rice samples. The peak areas were internal standard-corrected and the final peak areas normalized. In this way, the sample with the highest concentration of a particular analyte was assigned a relative value of 100% and all other sample concentrations were normalized as a fraction of this. The colors in the heat map represent the relative concentration with green and red corresponding to 0% and 100% respectively and various shades of yellow and orange in between.

The heat map allows direct visual comparison of the relative amounts of these compounds in the 12 samples tested.

The heat map shows that the relative concentration of the 66 metabolites analyzed varied widely among the 12 rice strains analyzed. In fact all of the metabolites studied are potential biomarkers and may be used to distinguish between different rice accessions. This quantitative metabolic data can be used with principal component analysis (PCA) to associate metabolic biomarkers with grain quality features of the contrasting rice.

The quantitation of these metabolites in the 12 rice samples was performed using the simultaneous Full Scan/SRM mode of the TSQ 8000, which provides the sensitivity and selectivity of SRM mode, required for accurate quantitation (Phase II—Targeted Analysis) in addition to enabling identification of new compounds from the full scan data (Phase I—Discovery). In order to assess the quality of the full scan data collected in simultaneous Full Scan/SRM mode, a NIST library search was used to identify unknowns. Figure 6 shows the chromatogram and corresponding mass spectrum for one of the samples analyzed.

The upper part of Figure 6 shows the total ion chromatogram (TIC) for the simultaneous Full Scan/SRM analysis of rice sample 13. The two black peaks at 30.65 and 30.78 minutes correspond to two compounds with SRM transitions developed using the AutoSRM optimization. The peak at 31.35 minutes is an unknown compound and as such had no SRM transition entered in the instrument method. The lower part of Figure 6 shows the full-scan spectrum for this unkmown compound. The spectrum was used to search the NIST library and identify the compound.

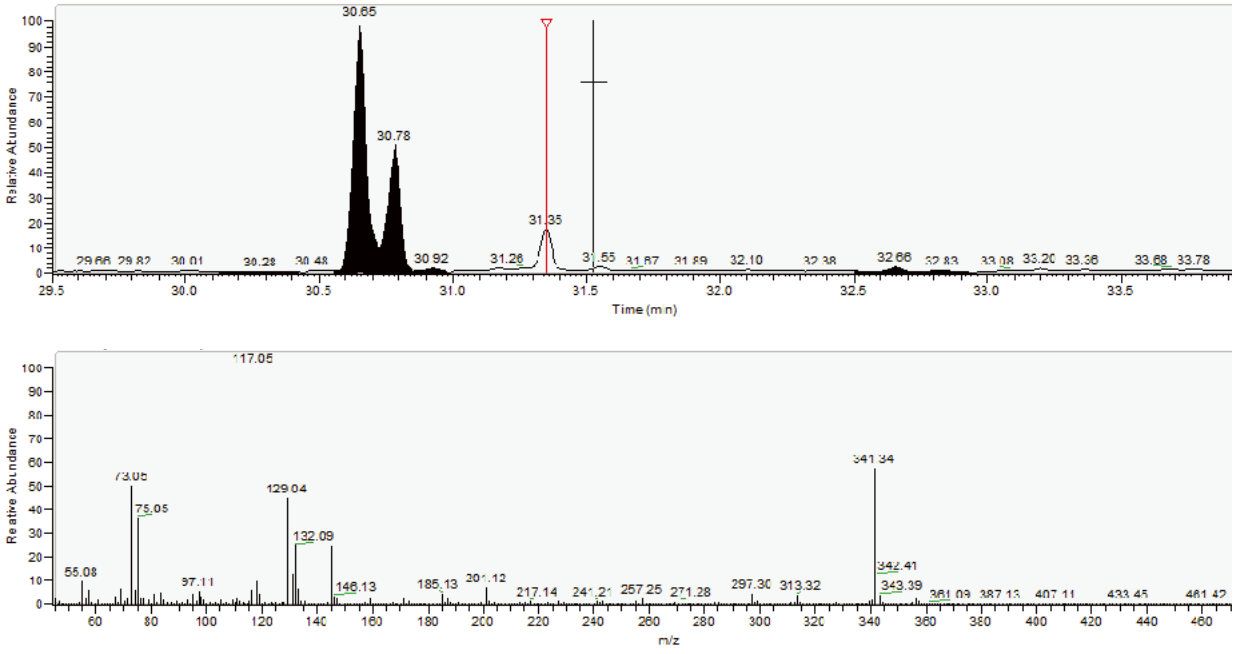

Figure 6. Total ion chromatogram (TIC), top, for simultaneous Full Scan/SRM analysis; black peaks are from SRM transitions. The mass spectrum, bottom, is the full scan spectrum of the unknown peak at 31.35 minutes.

Figure 7 shows the results of the NIST library search used to identify the unknown compound at 31.35 minutes using the full scan data collected during the simultaneous Full Scan/SRM analysis of rice sample 13. Figure 7 compares the unknown spectrum (red) with the NIST library spectrum for the best match (blue), octadecanoic acid-TMS ester. The library matched the unknown with a probability of 94.8%. The excellent quality of the data match illustrates the unique ability of the TSQ 8000 GC-MS/MS instrument to deliver library-searchable spectra during SRM analysis, thereby fulfilling the requirements of both Phase I (Discovery) and Phase II (Targeted) of the metabolomics workflow.

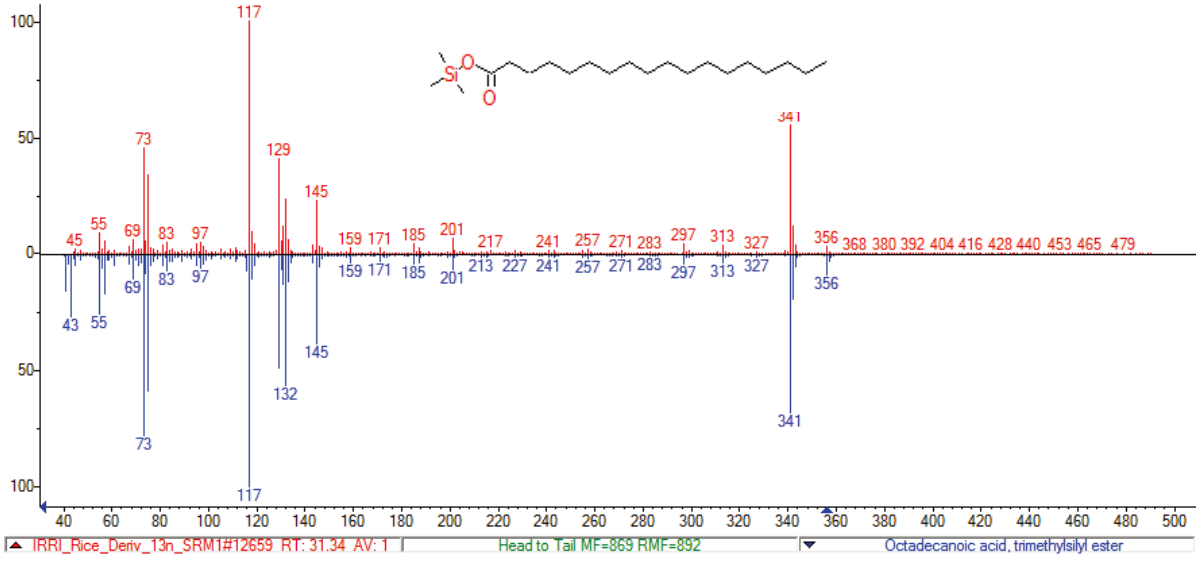

Figure 7. NIST library match for an unknown compound at 31.35 minutes, taken from simultaneous Full Scan/SRM analysis. Upper spectrum (red) is of unknown, lower spectrum (blue) is the NIST library match (octadecanoic acid-TMS ester).

Appendix 1 – Optimised SRM transitions for the 66 compounds selected in Phase I - Discovery.

			Quan Peak			Confirming Peak		
Compound Number	**Compound Name**	**RT (min)**	**Precursor**	**Product**	**CE (eV)**	**Precursor**	**Product**	**CE (eV)**
1	N,N'-Bis(trimethylsilyl)trifluoroacetamidine	6.37	99.0	69.0	30	99.0	71.0	10
2	Ethylbis(trimethylsilyl)amine	6.59	100.1	59.1	10	174.1	59.1	20
3	Silanamine, N,N'-methanetetraylbis[1,1,1-trimethyl-	6.92	78.1	64.0	12	78.1	71.3	12
4	1,2-Bis(trimethylsiloxy)ethane	7.44	148.8	45.0	30	148.8	75.0	10
5	Butane, 2,3-bis(trimethylsiloxy)-	8.73	118.4	45.6	18	118.4	75.2	8
6	l-Alanine, N-(trimethylsilyl)-, trimethylsilyl ester	10.06	116.1	43.0	28	116.1	45.1	18
7	Glycine, N-(trimethylsilyl)-, trimethylsilyl ester	10.48	102.1	45.1	20	102.1	58.0	30
8	Phosphoric acid, bis(trimethylsilyl)monomethyl ester	11.55	133.0	115.0	10	163.1	133.1	10
9	L-Valine, N-(trimethylsilyl)-, trimethylsilyl ester	12.32	144.1	43.0	32	144.1	58.1	32
10	2-(4-Methoxyphenyl)-2-(4-trimethoxysilyloxy)propane	13.36	299.1	151.1	10	300.5	74.1	10
11	Glycerol, tris(trimethylsilyl) ether	13.43	116.9	59.1	10	116.9	89.1	10
12	Butanedioic acid, bis(trimethylsilyl) ester	14.14	74.8	47.0	10	148.5	45.2	30
13	Serine, N,O-bis(trimethylsilyl)-, trimethylsilyl ester	14.94	205.5	205.3	10	218.2	45.1	30
14	N,O,O-Tris(trimethylsilyl)-L-threonine	15.37	100.6	86.1	10	117.1	43.0	30
15	Decanoic acid, trimethylsilyl ester	16.57	229.1	47.0	32	229.1	131.1	12
16	Butanedioic acid, [(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	17.04	189.7	147.1	10	233.1	73.1	10
17	Acetic acid, iodo-, trimethylsilyl ester	17.23	100.0	59.1	10	115.0	59.1	20
18	Threitol, 1,2,3,4-tetrakis-O-(trimethylsilyl)-, D-	17.35	205.3	117.1	8	205.3	147.1	8
19	L-Aspartic acid, N-(trimethylsilyl)-, bis(trimethylsilyl) ester	17.53	232.1	100.1	8	232.1	188.2	8
20	3-Trifluoromethylbenzylamine, N,N-dinonyl	17.73	171.1	73.1	22	314.1	73.1	22
21	Glutamic acid, N-(trimethylsilyl)-, bis(trimethylsilyl) ester, L-	19.12	128.1	73.1	10	246.1	73.1	20
22	Amine, N,N,N-tris((trimethylsilyloxy)ethyl)-	19.24	223.2	73.1	22	262.3	82.1	12
23	L-Asparagine, N,N2-bis(trimethylsilyl)-, trimethylsilyl ester	19.94	231.1	116.1	8	231.1	132.2	8
24	Arabinitol, pentakis-O-(trimethylsilyl)-	20.84	204.8	117.1	10	204.8	147.2	10
25	Phosphoric acid, bis(trimethylsilyl) 2,3-bis[(trimethylsilyl)oxy]propyl ester	21.59	299.2	147.2	20	299.2	225.2	10
26	l-Glutamine, tris(trimethylsilyl) deriv.	21.81	155.1	45.0	28	156.0	45.1	28
27	Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-	22.41	437.4	191.2	10	437.4	257.2	10
28	1,2,3-Propanetricarboxylic acid, 2-[(trimethylsilyl)oxy]-, tris(trimethylsilyl) ester	22.69	273.1	67.1	10	273.1	183.2	10
29	Tetradecanoic acid, trimethylsilyl ester	23.30	285.2	95.2	12	285.2	131.1	12
30	β -Hydroxypyruvic acid, trimethylsilyl ether, trimethylsilyl ester	23.86	518.5	188.3	12	518.5	428.3	12
31	Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, D-	24.34	191.1	45.1	30	191.1	147.2	10
32	N,O,O'-Tris-(trimethylsilyl)tyrosine	25.02	218.1	73.1	8	218.1	100.1	8
33	Hexadecanoic acid, trimethylsilyl ester	27.31	73.1	45.1	12	73.1	58.0	22
34	Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	28.15	305.2	147.2	8	305.2	217.2	8
35	Tryptophan, bis(trimethylsilyl)-	30.27	202.1	45.1	32	202.1	58.0	32
36	9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	30.70	81.0	79.1	10	337.2	131.1	10
37	Oleic acid, trimethylsilyl ester	30.82	339.5	75.1	20	339.5	131.1	10
38	α -L-Galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl)-	32.65	146.9	45.0	30	146.9	131.2	10
39	2-Pyrrolidinone, 1-(9-octadecenyl)-	32.78	85.0	43.9	12	98.1	55.1	12
40	Myristic acid, 2,3-bis(trimethylsiloxy)propyl ester	34.40	211.2	71.1	10	343.5	95.1	10
41	Myo-Inositol, pentakis-O-(trimethylsilyl)-, bis(trimethylsilyl) phosphate	34.64	318.4	147.2	20	318.4	215.2	10
42	β -D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic methylboronate	34.73	225.1	79.1	10	225.1	93.1	10
43	Eicosanoic acid, trimethylsilyl ester	35.27	117.0	47.1	20	117.0	75.1	10
44	α -D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)- β -D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	38.00	147.1	45.1	32	147.1	131.1	12
45	Sucrose, octakis(trimethylsilyl) ether	39.09	362.7	169.2	10	362.7	170.2	10
46	D-(+)-Trehalose, octakis(trimethylsilyl) ether	40.81	361.4	169.1	10	361.4	243.3	10
47	9-Octadecenoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester	41.00	103.0	45.1	20	103.0	58.1	30
48	β -D-Xylopyranose, 1,2,3,4-tetrakis-O-(trimethylsilyl)-	41.54	399.3	81.1	10	399.3	95.5	10
49	1,2-Propanediol-1-phosphate, tris(trimethylsilyl)-	42.68	298.9	147.1	20	298.9	225.2	10
50	D-Turanose, heptakis(trimethylsilyl)- Isomer 1	42.81	373.4	167.1	12	373.4	211.2	12
51	D-Turanose, heptakis(trimethylsilyl)- Isomer 2	43.00	361.1	169.2	10	361.1	243.3	10
52	β -D-Xylopyranose, 1,2,3,4-tetrakis-O-(trimethylsilyl)	43.67	147.2	45.1	32	147.2	131.1	12
53	α -D-Galactopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	44.71	204.0	73.1	10	204.0	189.2	10
54	β -D-Xylopyranose,1,2,3,4-tetrakis-O-(trimethylsilyl)-	44.81	147.3	131.1	10	129.3	45.0	20
55	α -D-Galactopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl)- Isomer 1	45.01	488.7	222.8	20	488.7	223.5	10
56	1,2-Propanediol-1-phosphate, tris(trimethylsilyl)- isomer 1	45.85	357.1	225.1	18	357.1	341.2	8
57	D-Glucose, 4-O-[2,3,4,6-tetrakis-O-(trimethylsilyl)- β -D-galactopyranosyl]-2,3,5,6-tetrakis-O-(trimethylsilyl)-	46.57	205.2	45.3	30	205.2	190.1	10
58	2-Methyl-2(p-methoxy)mandelate, bis(trimethylsilyl)-	46.81	222.0	45.1	32	222	194.1	12
59	1,2-Propanediol-1-phosphate, tris(trimethylsilyl)- isomer 2	48.44	211.0	115.1	30	211	133.1	10
60	Silane, [[(3 β ,24R)-ergost-5-en-3-yl]oxy]trimethyl-	48.84	343.2	95.2	20	343.2	121.1	10
61	β -Sitosterol trimethylsilyl ether	50.16	357.3	95.1	20	357.3	107.1	20
62	α -D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)- β -D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	51.24	362.5	169.2	12	362.5	170.2	12
63	9,19-Cyclolanostan-3-ol, 24-methylene-, (3 β)-	52.13	147.0	105.1	10	147.0	119.1	10
64	D-Turanose, heptakis(trimethylsilyl)- isomer 3	53.49	217.2	45.1	28	361.2	169.2	8
65	2-O-Glycerol- α -d-galactopyranoside, hexa-TMS isomer 1	54.95	217.1	45.1	32	217.1	143.1	12
66	D-Glucose, 4-O-[2,3,4,6-tetrakis-O-(trimethylsilyl)- β -D-galactopyranosyl]-2,3,5,6-tetrakis-O-(trimethylsilyl)-	59.21	204.1	45.1	30	204.1	189.2	10

Conclusions

The Thermo Scientific TSQ 8000 was used for this primary metabolite profiling study of rice genotypes provided by the International Rice Research Institute, with the aim of identifying potential biomarkers for characterization in support of grain quality research. The instrument performed extremely well for both Phase I (Discovery) and Phase II (Targeted Quantitation) of the metabolomics workflow and demonstrated the unique advantages of this instrument in both full scan, SRM, and simultaneous Full Scan/SRM modes for both profiling and quantification of wide range of primary metabolites.

Although the TSQ 8000 instrument is a triple quadrupole analyzer, the acquired spectra in full scan mode showed excellent agreement with the mass spectra of the NIST spectral library. The identification of unknown compounds could be achieved by library search with high confidence. Isomeric compounds have been distinguished on the basis of their elution at differing retention times, and could be selectively quantified based on their specific fragmentation profiles.

The AutoSRM software package of the TSQ 8000 was used to develop and optimize a SRM method for 66 metabolites in the rice samples, including amino, organic and fatty acids, alcohols, and sugars (mono-, di-saccharides). The optimized SRM method was completed in less than 24 hours and used to analyze these metabolites in 12 diverse rice samples. The quantitative results showed excellent reproducibility with RSDs < 15% for more than 90% of the metabolites, even in the high matrix of the derivatized rice extracts.

All metabolites investigated varied significantly among rice samples and therefore have potential for use as biomarkers for the characterization of diverse rice accessions. This work forms an excellent basis for future studies to tap in the value of diversity to improve grain quality and nutritional value of rice in the larger panels.

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