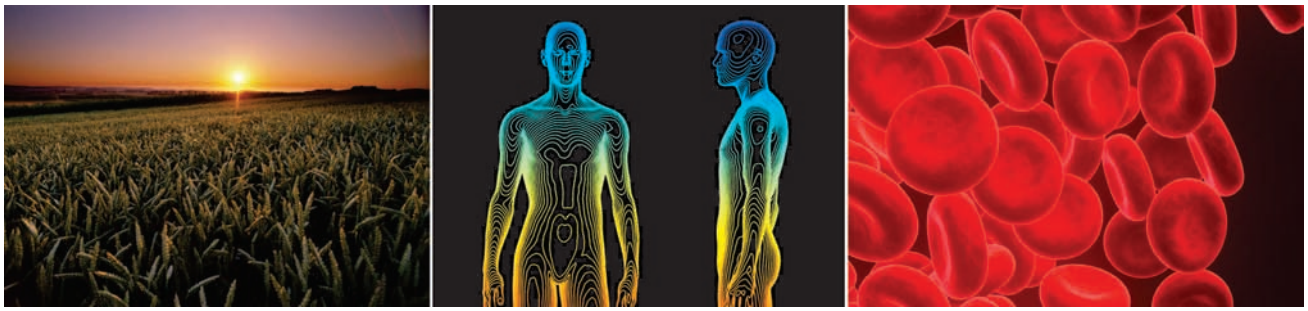

Metabolomic Profiling of Corn and Rice Extracts Using a Benchtop GC-TOFMS

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Key Words: GC-TOFMS, Corn, Rice, Derivatization, High Throughput, Quantitation, Metabolomics, Amino Acids, Deconvolution

1. Introduction

Corn and rice are some of the most important crops worldwide for food, feed, and energy.[1,2] Metabolomic profiling is one of the most effective approaches for studying the general characteristics of rice and corn, their resistance to insects, and their tolerance to stressful environmental conditions.[3] Metabolomics can be used to study crop diversity and disease resistance. These studies often lead to an improvement in quality, yield, and nutritional value.[4,5] More specifically, food quality traits such as fragrance, taste, appearance, shelf-life, and nutritional content can be assessed by determining their biochemical content.[6] In this study, we took advantage of a new benchtop GC-TOFMS for metabolomics profiling of corn and rice extracts. Instrument parameters were optimized for speed and detection of a wide array of metabolites after samples were derivatized via silylation. Powerful software tools were used to acquire, peak find, and quickly annotate the rich, high quality spectral data produced by LECO Corporation's new Pegasus® BT mass spectrometer.

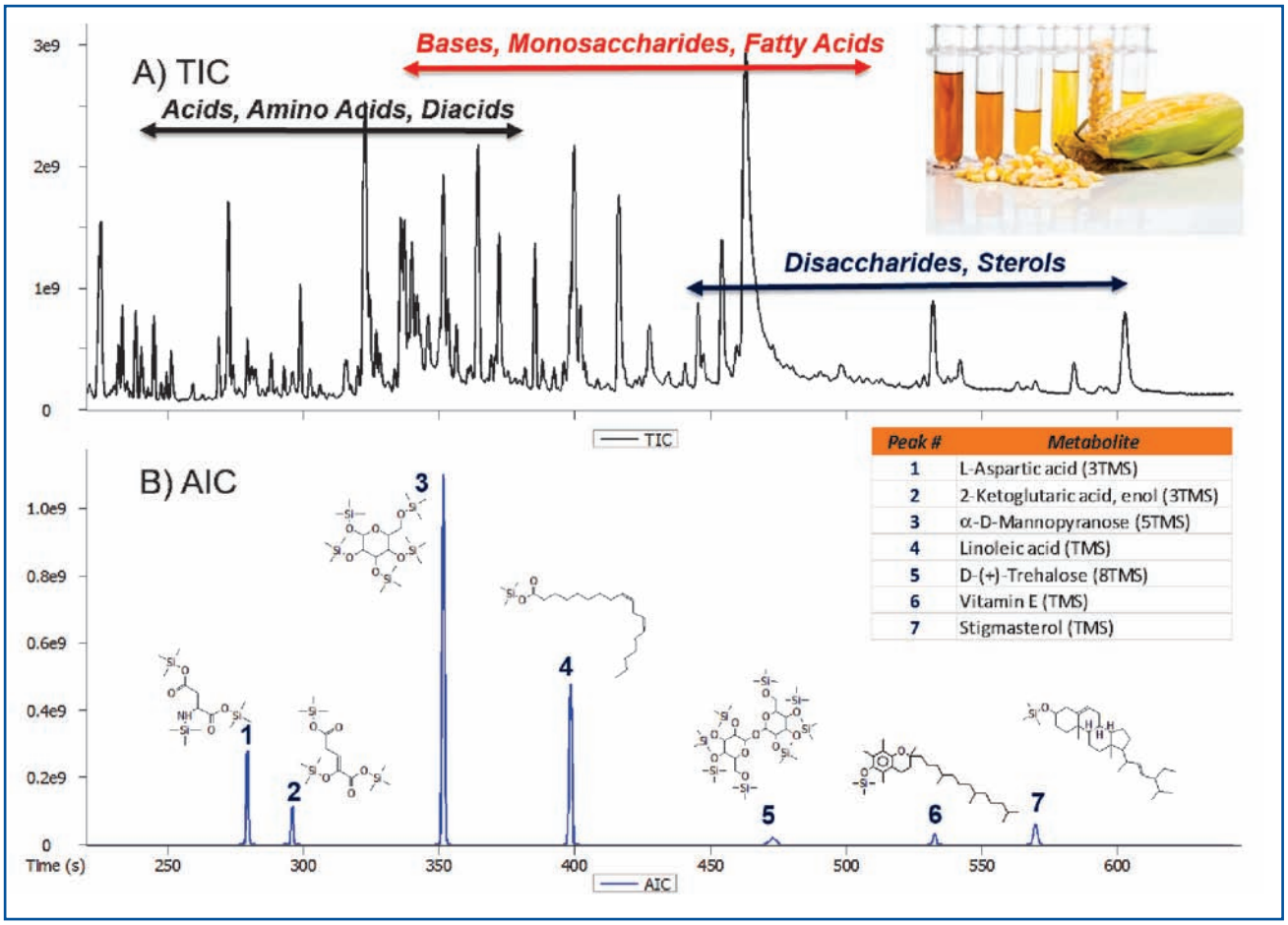

Figure 1. Total Ion Chromatogram (A) and Analytical Ion Chromatogram (B) showing the ability to selectively determine analytes of interest in a complex corn leaf extract.

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2. Experimental

a) Extraction

400 µL of 4:1 methanol/water was added to 25 mg of freeze-dried plant material. The heterogeneous mixture was vortexed for 5 minutes, heated at 70°C for 15 minutes, and centrifuged for 5 minutes at 12,000 rpm. Ribitol was added as an internal standard (10 µL; 2000 µg/mL in water) to 400 µL of supernatant. The resulting mixture was dried under vacuum at 60°C using a Speed Vac.

b) Derivatization

Dry samples were derivatized by adding 100 µL of MSTFA and heating at 60°C for 1 hour.

Table 1. GC- TOFMS (*Pegasus* BT) Conditions

Gas Chromatograph	**Agilent 7890 with GERSTEL MPS2 Autosampler**
Injection	1 µL, split 20:1 @ 270°C
Carrier Gas	He @ 0.80 mL/min , Constant Flow
Column	Rxi-5ms, 20 m x 0.18 mm i.d. x 0.18 µm coating (Restek, Bellefonte, PA, USA)
Oven Program	60°C (0.5 min), to 320°C @ 36°C/min (3 min)
Transfer Line	300°C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250°C
Mass Range	45-600 m/z
Acquisition Rate	20 spectra/s

3. Results and Discussion

The *Pegasus* BT is a benchtop instrument with the expected characteristics of a state-of-the-art GC-TOFMS (i.e., speed, robustness, chromatographic resolution, etc.), but with improved sensitivity (parts-per-trillion) and extended linear dynamic range (10^5). This is clearly illustrated for a set of amino acids in a calibration range from 0.01 to 50 pMol/µL (Table 2). Linearity for calibration curves was excellent as evident from the correlation coefficients for methionine, phenyl alanine aspartic acid, and lysine which ranged from 0.996 to 0.998 (Figure 2). An expansion of the lower portion of the curves for branched amino acids Leucine and Isoleucine demonstrates that linearity is maintained throughout the calibration range (Figure 3; r = 0.999).

Table 2. Amino Acid Standard Concentrations are shown in both pMol/µL (0.01 to 50 pMol/µL) and pg on column after a 1 µL injection, split 20:1.

Amino Acid	pg On Column after a 1uL Injection/Split 20:1								
Ala	223	111	45	22	4.5	2.2	0.45	0.22	0.045
Arg	436	218	87	44	8.7	4.4	0.87	0.44	0.087
Asp	333	166	67	33	6.7	3.3	0.67	0.33	0.067
Cys	303	151	61	30	6.1	3.0	0.61	0.30	0.061
Glu	368	184	74	37	7.4	3.7	0.74	0.37	0.074
Gly	188	94	38	19	3.8	1.9	0.38	0.19	0.038
His	388	194	78	39	7.8	3.9	0.78	0.39	0.078
Ile	328	164	66	33	6.6	3.3	0.66	0.33	0.066
Leu	328	164	66	33	6.6	3.3	0.66	0.33	0.066
Lys	365	183	73	37	7.3	3.7	0.73	0.37	0.073
Met	373	187	75	37	7.5	3.7	0.75	0.37	0.075
Phe	413	206	83	41	8.3	4.1	0.83	0.41	0.083
Pro	288	144	58	29	5.8	2.9	0.58	0.29	0.058
Ser	263	131	53	26	5.3	2.6	0.53	0.26	0.053
Thr	298	149	60	30	6.0	3.0	0.60	0.30	0.060
Tyr	453	226	91	45	9.1	4.5	0.91	0.45	0.091
Val	293	146	59	29	5.9	2.9	0.59	0.29	0.059
Conc. pMol/µL	***50***	***25***	***10***	***5***	***1***	***0.5***	***0.1***	***0.05***	***0.01***

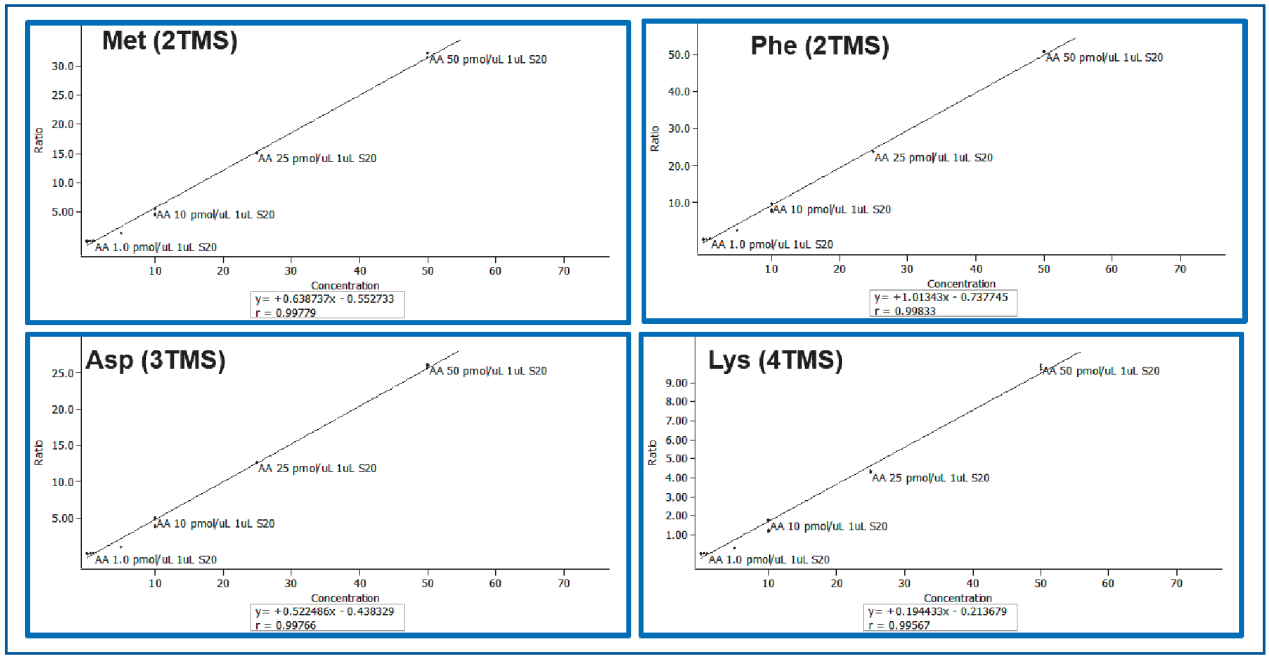

Figure 2. Calibration Curves for Methionine, Phenyl Alanine, Aspartic Acid, and Lysine (0.01 to 50 pMol/µL).

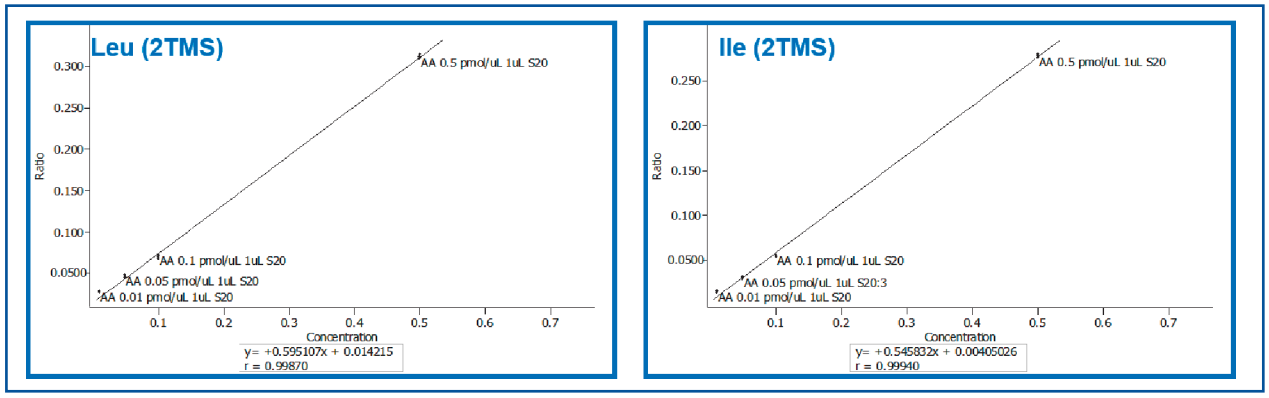

Figure 3. Expansion of Calibration Curves for Leucine and Isoleucine (0.01 to 0.5 pMol/µL).

The combination of LECO's high-performance hardware and next generation ChromaTOF® brand software (version 5.0) resulted in an increased coverage of annotated metabolites in corn and rice samples. Comprehensive metabolomics data was acquired in just over 10 minutes. Metabolites were confidently identified via automated, comprehensive data processing which involved peak find, spectral similarity searches against large well-established databases, and by leveraging retention index values. A set of forty-two representative metabolites detected and annotated in corn extract are listed in Table 3. These compounds include amino acids, acids, diacids, monosaccharides, disaccharides, terpenes, terpenoids, and sterols. The metabolites were compared against NIST and Wiley databases and found to have an average spectral similarity score of 870/1000. The quality and richness of data produced by the *Pegasus* BT is nicely illustrated by the spectral similarity comparisons of Peak True (Deconvoluted) and NIST database mass spectra for (E)-aconitic acid and palmitic acid, which were 916 and 943/1000, respectively (Figure 4).

Peak #	Name	Formula	R.T. (s)	Retention Index	Area	Similarity
1	Isoleucine 2TMS	$C_{12}H_{29}NO_2Si_2$	230	1291	395477933	814
2	Niacin TMS	$C_9H_{13}NO_2Si$	230	1294	108299128	870
3	Malonic Acid 2TMS	$C_9H_{20}O_4Si_2$	231	1298	1436217142	876
4	Succinic 2TMS	$C_{10}H_{22}O_4Si_2$	233	1306	2251723198	854
5	Glyceric Acid 3TMS	$C_{12}H_{30}O_4Si_3$	238	1328	1516160071	929
6	Itaconic Acid 2TMS	$C_{11}H_{22}O_4Si_2$	240	1340	2239319374	918
7	Methylmaleic Acid 2TMS	$C_{11}H_{22}O_4Si_2$	242	1351	992776815	805
8	Serine 3TMS	$C_{12}H_{31}NO_3Si_3$	244	1361	1484484365	909
9	(E)-Erythrono-1,4-lactone 2TMS	$C_{10}H_{22}O_4Si_2$	249	1384	1013322110	937
10	Threonine 3TMS	$C_{13}H_{33}NO_3Si_3$	251	1392	2342030066	905
11	Methylmaleic Acid 2TMS	$C_{11}H_{22}O_4Si_2$	252	1396	857642597	865
12	Malic Acid 3TMS	$C_{13}H_{30}O_5Si_3$	272	1496	3562505366	874
13	Threonic Acid 4TMS	$C_{16}H_{40}O_5Si_4$	288	1572	2221809977	947
14	2-Ketoglutaric Acid 3TMS	$C_{14}H_{30}O_5Si_3$	296	1610	1425602596	871
15	Pyrogallol 3TMS	$C_{15}H_{30}O_3Si_3$	297	1615	70015660	804
16	Glutamic Acid 3TMS	$C_{14}H_{33}NO_4Si_3$	299	1627	1737232874	853
17	L-Rhamnose 4TMS	$C_{18}H_{44}O_5Si_4$	302	1644	1956202287	830
18	(Z)-Aconitic Acid 3TMS	$C_{15}H_{30}O_6Si_3$	322	1754	5787376222	923
19	Neophytadiene	$C_{20}H_{38}$	339	1846	330374033	931
20	Glucopyranose 5TMS	$C_{21}H_{52}O_6Si_5$	352	1923	3685576368	904
21	Mannose 5TMS	$C_{21}H_{52}O_6Si_5$	352	1923	3164023678	810

Peak #	Name	Formula	R.T. (s)	Retention Index	Area	Similarity
22	Glucose 5TMS	$C_{21}H_{52}O_6Si_5$	365	1999	2193633580	912
23	Palmitic Acid TMS	$C_{19}H_{40}O_2Si$	372	2050	2740753179	943
24	D-Gluconic acid 6TMS	$C_{24}H_{60}O_7Si_6$	374	2057	285082038	828
25	Ferulic Acid 2TMS	$C_{16}H_{26}O_4Si_2$	382	2110	351778560	853
26	Myo-Inositol 6TMS	$C_{24}H_{60}O_6Si_6$	385	2133	1907082179	824
27	Caffeic Acid 3TMS	$C_{18}H_{32}O_4Si_3$	388	2152	1090954337	885
28	Phytol TMS	$C_{23}H_{48}OSi$	393	2181	1688201136	913
29	Stearic Acid TMS	$C_{21}H_{44}O_2Si$	402	2246	970175017	869
30	Tryptophan 3TMS	$C_{20}H_{36}N_2O_2Si_3$	404	2258	336310682	811
31	2-O-Glycerol galactopyranoside 6TM	$C_{27}H_{66}O_8Si_6$	416	2347	3540461506	906
32	Glucose 6-Phosphate 6TMS	$C_{24}H_{61}O_9PSi_6$	426	2414	486549797	803
33	Myo-Inositol Phosphate 7TMS 7	$C_{27}H_{69}O_9PSi_7$	434	2475	525744735	902
34	Adenosine 5'-Phosphate 5TMS	$C_{25}H_{54}N_5O_7PSi_5$	520	3132	66380886	776
35	α-Tocopherol TMS	$C_{32}H_{58}O_2Si$	533	3228	575984929	903
36	3-O-Feruloylquinic Acid 5TMS	$C_{32}H_{60}O_8Si_5$	540	3279	683024379	820
37	Chlorogenic Acid 6TMS	$C_{34}H_{66}O_9Si_6$	543	3298	3292317512	779
38	Mannobiose 8TMS	$C_{36}H_{86}O_{11}Si_8$	567	3479	2142914778	850
39	Stigmasterol TMs	$C_{32}H_{56}OSi$	570	3501	482314345	843
40	3-hydroxystigmast-5-ene TMS	$C_{32}H_{58}OSi$	585	3608	1079446063	918
41	α-Amyrin TMS	$C_{33}H_{58}OSi$	594	3678	305914381	888
42	Sucrose 8TMS	$C_{36}H_{86}O_{11}Si_8$	600	3718	1779889280	885

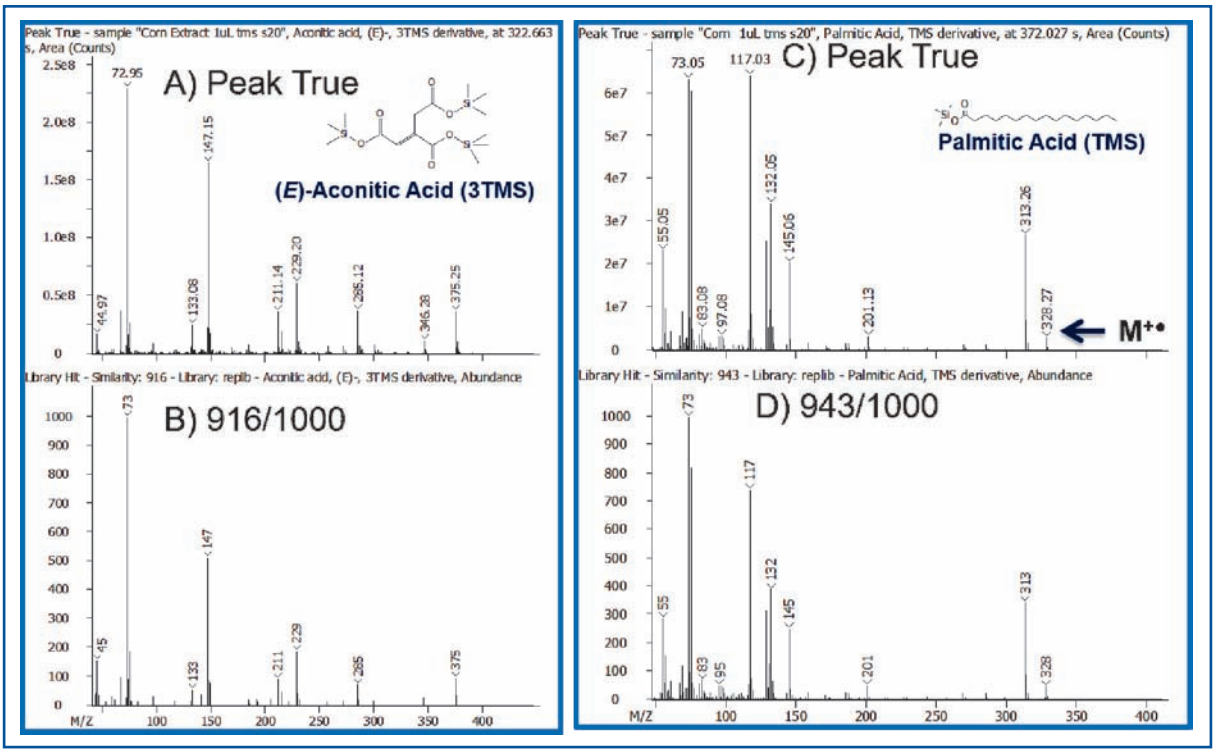

Figure 4. Comparison of Peak True (Deconvoluted-Top) vs. Library Spectra (Bottom) for (E)-Aconitic Acid and Palmitic Acid.

An extra level of confidence in metabolite identification was achieved by combining database search results with retention index (RI) values as shown for serine and phytol in Figure 5. The similarity scores for serine and phytol were 966 and 945/1000, and the retention index values were 1361 (Reference value = 1369) and 2179 (Reference value = 2181) respectively.

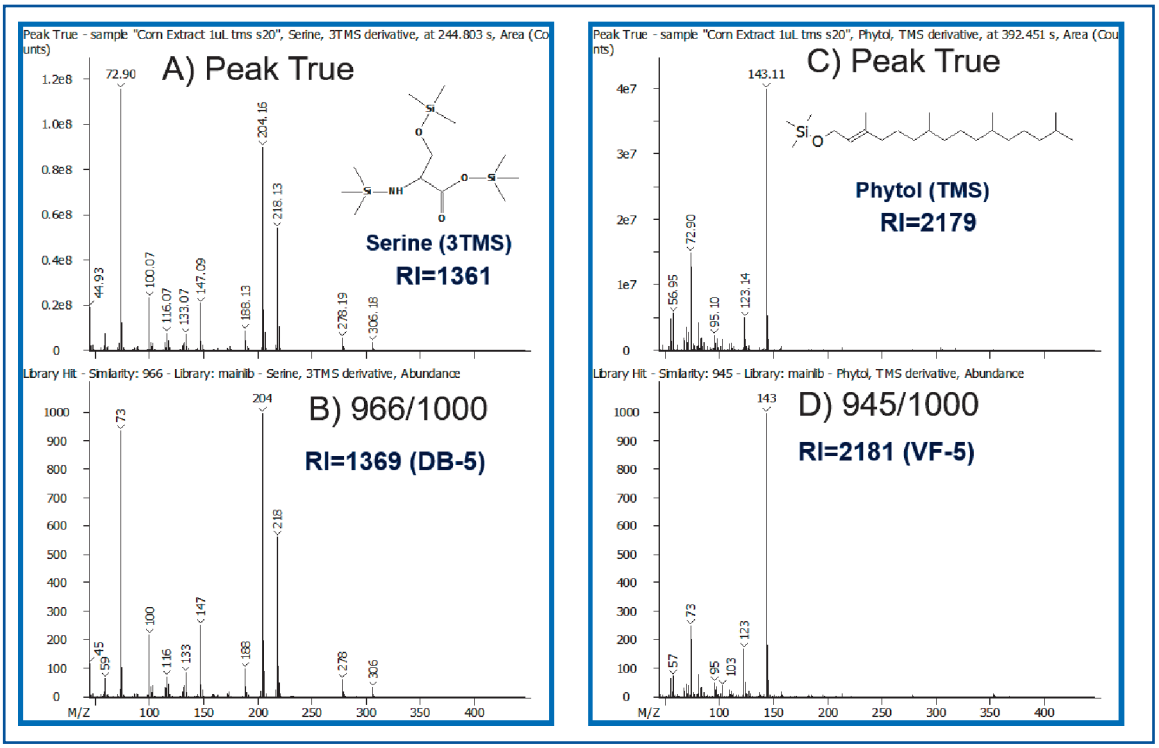

Figure 5. Comparison of Peak True (Deconvoluted-Top) vs. Library Spectra (Bottom) for Serine and Phytol. Calculated RI values are also shown.

A very exciting feature of *ChromaTOF* 5.0 is the ability to process comprehensive data retrospectively in a targeted manner. Target Analyte Finding (TAF) is ideal for trace analysis, quantitative analysis, and processing large datasets. TAF significantly reduces processing times by leveraging retention times, fragment and/or molecular ions, and mass tolerances (Figure 6). A total of 22 metabolites were targeted in the corn and rice data files (Figures 7 and 8). TAF results can be easily exported during processing for further analysis (e.g., statistical).

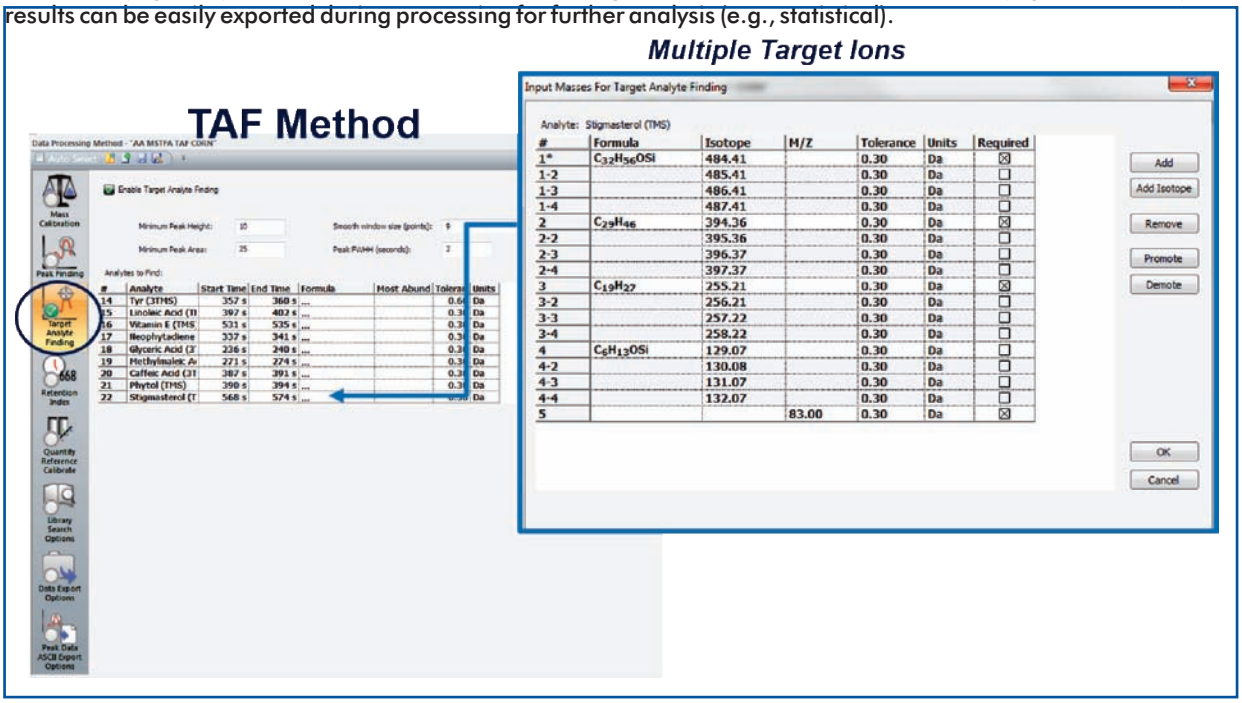

Figure 6. ChromaTOF 5.0 Target Analyte Finding Processing (TAF) Method.

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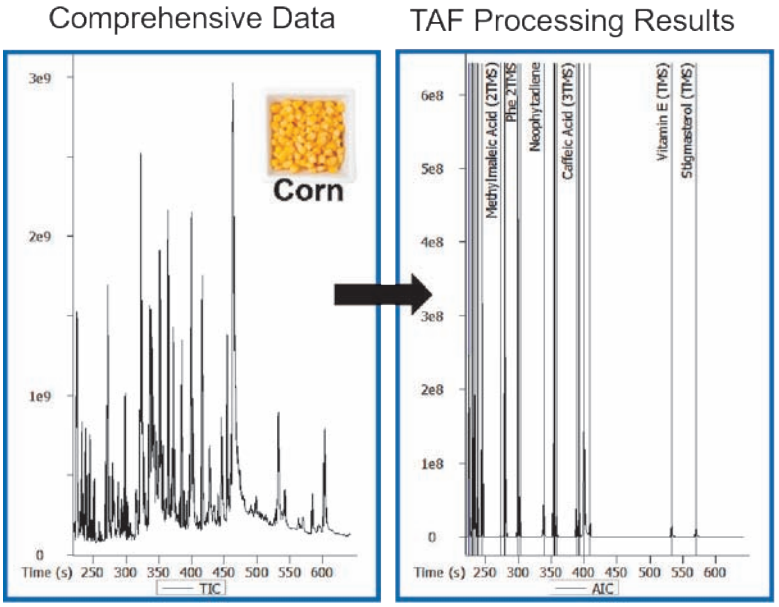
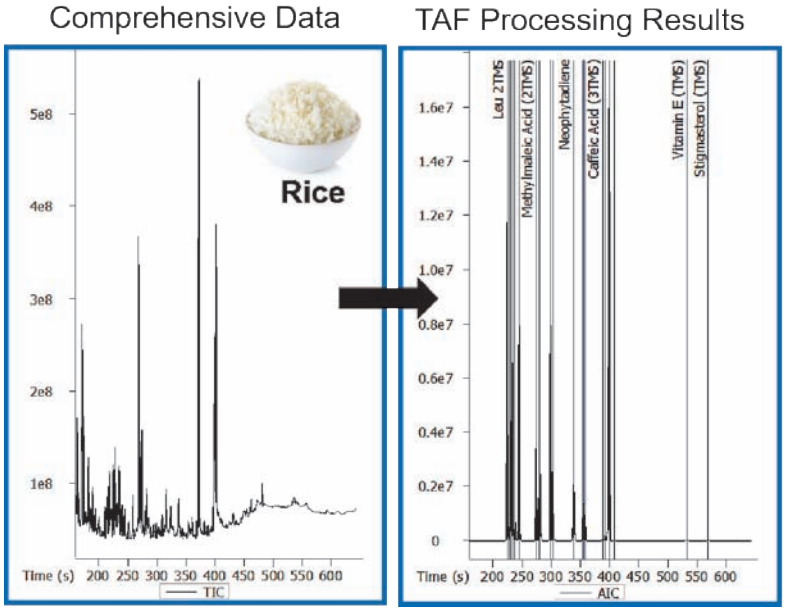


Name	R.T. (s)	Area
Leu 2TMS	225	95603199
Ile 2TMS	230	105295635
Pro 2TMS	232	386416905
Gly 3TMS	234	100883766
Glyceric Acid 3TMS	238	468854667
Ser 3TMS	245	738788832
Methylmaleic Acid 2TMS	272	7244266
Asp 3TMS	279	524066535
Met 2TMS	280	24261207
Glu 3TMS	299	1044206029
Arg 5TMS	299	16282129
Phe 2TMS	302	81723525
Neophytadiene	339	316422034
Lys 4TMS	355	17964039
His 3TMS	355	20172491
Tyr 3TMS	358	152760014
Caffeic Acid 3TMS	388	328176924
Phytol TMS	393	344592658
Linoleic Acid TMS	400	2065982651
Cys 3TMS (d)	409	15628784
Vitamin E TMS	533	206965487
Stigmasterol TMS	570	154103044

Figure 7. TAF Processing Results – Corn Extract.



Name	R.T. (s)	Area
Leu 2TMS	224	20052204
Ile 2TMS	230	14272493
Pro 2TMS	231	14715420
Gly 3TMS	233	54347435
Glyceric Acid 3TMS	238	11405111
Ser 3TMS	245	57093519
Methylmaleic Acid 2TMS	274	5494074
Asp 3TMS	279	25101891
Met 2TMS	280	1714524
Glu 3TMS	299	19914482
Arg 5TMS	299	375792
Phe 2TMS	302	8608225
Neophytadiene	338	40709759
Lys 4TMS	354	1040632
His 3TMS	354	885995
Tyr 3TMS	358	7400350
Caffeic Acid 3TMS	388	2325703
Phytol TMS	392	4394761
Linoleic Acid TMS	399	572738853
Cys 3TMS (d)	408	121028
Vitamin E TMS	532	729919
Stigmasterol TMS	569	888793

Figure 8. TAF Processing Results – Rice Extract.

4. Conclusion

LECO's *Pegasus* BT is a powerful and robust benchtop mass spectrometer with excellent sensitivity and extended linear dynamic range. The combination of the *Pegasus* BT instrument and next generation *ChromaTOF* brand software resulted in the production of high-quality data that could be processed rapidly using Peak Find and/or retrospectively with Target Analyte Finding. The *Pegasus* BT is an indispensable tool for quick, effective, and routine analysis of complex biological samples.

5. References

[1]M. Chen, R.S.P. Rao, Y. Zhang, C. Zhong and J.J. Thelen, *The Crop Journal*, **2016**, 4, 177-187.

[2]M. Kusano, Z. Yang, Y. Okazaki, R. Nakabayashi, A. Fukushima and K. Saito, *Molecular Plant*, **2015**, 8, 58-67.

[3]Y. Okazaki and K. Saito, *Plant Biotechnol. Rep.*, **2012**, 6, 1-15.

[4]C. Simo, C. Ibanez, A. Valdes, A. Cifuentes and V. Garcia-Canas, *Int. J. Mol. Sci.*, **2014**, 15, 18941-18966.

[5]G. Astarita nad J. Langridge, *Journal of Nutrigenetics and Nutrigenomics,* **2013**, 6, 181-200.

[6]J.W. Allwood, A. Erban, S. De Koning, W.B. Dunn, A. Luedemann, A. Lommen, L. Kay, R. Loscher, J. Kopka, and R. Goodacre, *Metabolomics*, **2009**, 5, 479-496.

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