Application Note

Instrument: Pegasus® GC-HRT 4D

Utilizing the Pegasus GC-HRT 4D for Improved Yeast Metabolite Characterization

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Key Words: Yeast, Metabolomics, Gas Chromatography, High Resolution Mass Spectrometry

Introduction

Yeast has been used since ancient times for fermentation and production of wine.[1] In modern times, the S. cerevisiae strain is the preferred cell workhorse for the production of bioethanol.[2] Yeast also continues to serve as a model for systems biology and is being studied as a potential "cellular factory" for production of additional sustainable chemicals such as valuable organic acids.[3,4] In order for this bioengineering to become realized, the metabolism of yeast must be clearly understood. The biggest bottleneck in metabolomics research and engineering is characterization of the diverse classes of compounds found in living systems. In this study, we explored the utilization of gas chromatography—high resolution time of flight mass spectrometry (GC-HRT) and comprehensive two-dimensional gas chromatography—high resolution time of flight mass spectrometry (GCxGC-HRT) for molecular profiling of yeast. Combining the strengths of GCxGC (increased peak capacity, chromatographic resolution, and the ability to provide cleaner spectra) with high resolution and accurate mass capabilities, the *Pegasus* GC-HRT 4D allows researchers to take their metabolomics discovery workflows to the next level. Figure 1 clearly demonstrates the benefit of increased chromatographic resolution for complex metabolomic samples.

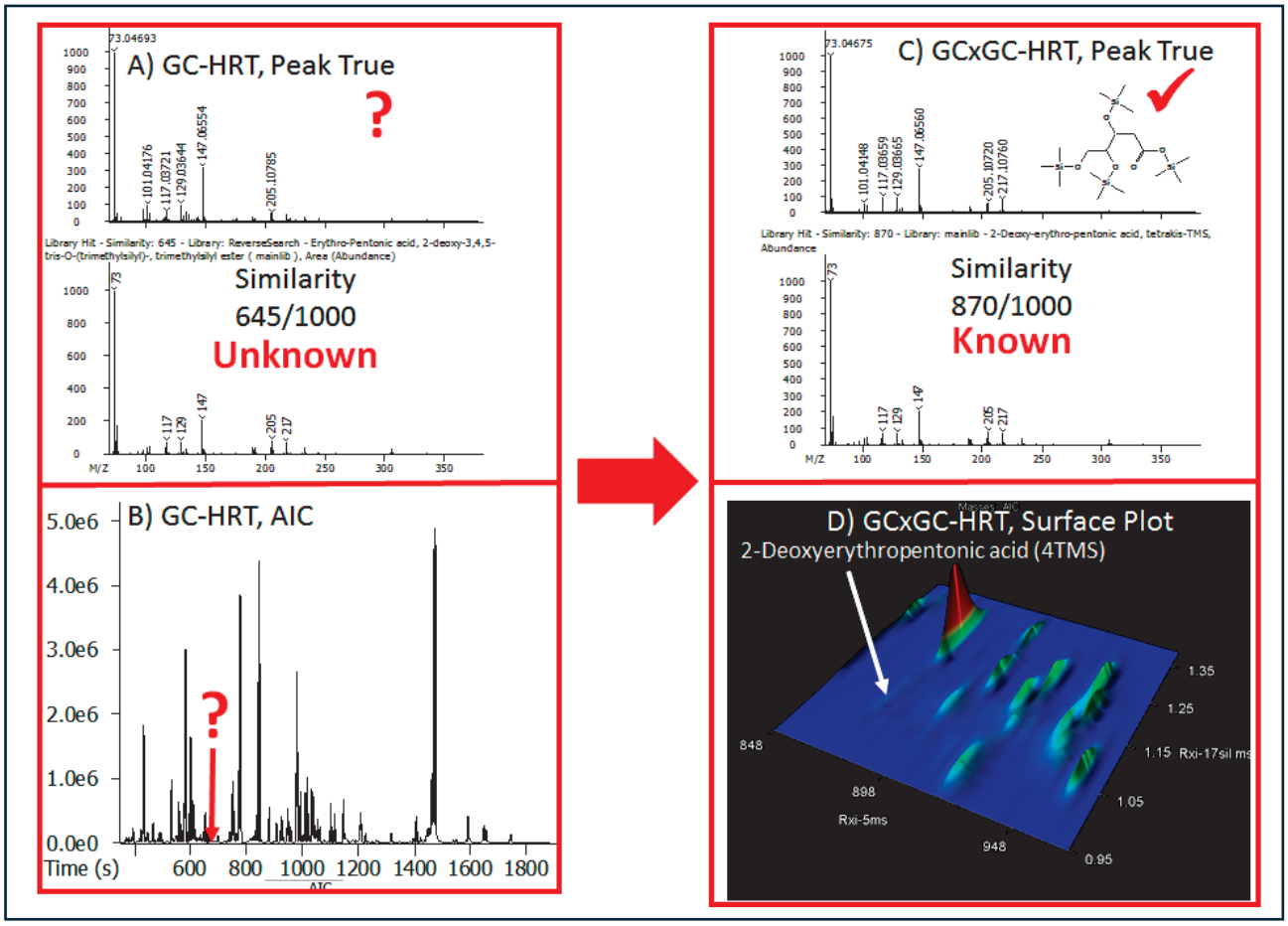


Figure 1. GC-HRT Peak True Mass Spectrum and AIC (A, B) vs. GCxGC-HRT Mass Spectrum and Surface Plot (C, D) for Characterization of 2-Deoxyerthropentonic Acid (4TMS).

Experimental

Yeast autolysate (Sigma-Aldrich) and dry Baker's yeast (local supermarket) were prepared for analysis using the following protocol. Yeast powder (15-30 mg) was extracted with 10 mL 1:1:2 $H_2O/CH_3OH/CHCl_3$ and filtered into a 2 mL GC vial. The solvents were removed using a Speed Vac. The residue was then derivatized using a two-step procedure: 1) Methoximation with 20 µL of MEOX (20 mg/mL methoxylamine hydrochloride in pyridine, 60 minutes at 60°C) followed by 2) treatment with 80 µL of MSTFA (60 minutes at 60°C).

Both GC and GCxGC data were collected using the instrumental parameters detailed in Table 1. Comprehensive data processing was accomplished using ChromaTOF® brand software: 1) Mass calibration, 2) Peak Find (Deconvoluted data), and 3) spectral similarity searches using NIST 2014 and Wiley 10.

Table 1. *Pegasus* HRT GC-High Resolution TOFMS Conditions

Gas Chromatograph	**LECO GCxGC Quad Jet Thermal Modulator & MPS2 Autosampler**
Injection	1 µL, Split 20:1 @ 250°C; (Splitless for CI)
Carrier Gas	He @ 1.0 ml/min, Constant Flow
Column One	Rxi-5 MS, 30 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA)
Column Two	Rxi-17SilMS, 0.9 m x 0.25 mm x 0.25 µm coating (Restek, Bellefonte, PA, USA)
Temperature Program	1 min at 65°C, ramped 10°C/min to 320°C, held 6 min Secondary oven maintained +5°C relative to primary oven
Modulation	1D acquisitions: 0s 2D acquisitions: 3s *Modulator temperature maintained +15°C relative to 2nd oven
Mass Spectrometer	**LECO *Pegasus* HRT**
Transfer Line	300°C
Ion Source Temperature	250°C (EI); 200°C (CI)
Acquisition Mode	High Resolution, R = 25,000 (FWHM)
Ionization Mode	EI and or CI (Reagent Gas: 5% NH_3 in CH_4)
Mass Range (m/z)	35-510 (EI); 60-1200 (CI)
Acquisition Rate	GC-HRT: 10 spectra/s; GCxGC-HRT: 200 spectra/s

Results and Discussion

A yeast autolysate sample, spiked with twenty-three D and ^{13}C isotopically labeled standards, was extracted and analyzed by gas chromatography—high resolution time-of-flight mass spectrometry (GC-HRT) operating at a resolving power of 25,000 (m/z = 219). In addition, complementary EI and CI ionization methods, spectral similarity searches of large databases (NIST, Wiley), and formulae determinations for accurate mass (<1 ppm) ions were applied for confident characterization of yeast compounds. The results of High Resolution Deconvolution® (HRD®) are characterized in the analytical ion chromatogram (AIC) for a yeast extract sample which is shown in Figure 2. Table 2 lists the names, formulas, retention times, areas, and similarity values for a representative set of yeast components. These compounds included acids, diacids, amino acids, sugars, bases, nucleosides, and nucleotides. Spectral similarity searches against the NIST and Wiley libraries resulted in an average similarity of 911/1000 for the set.

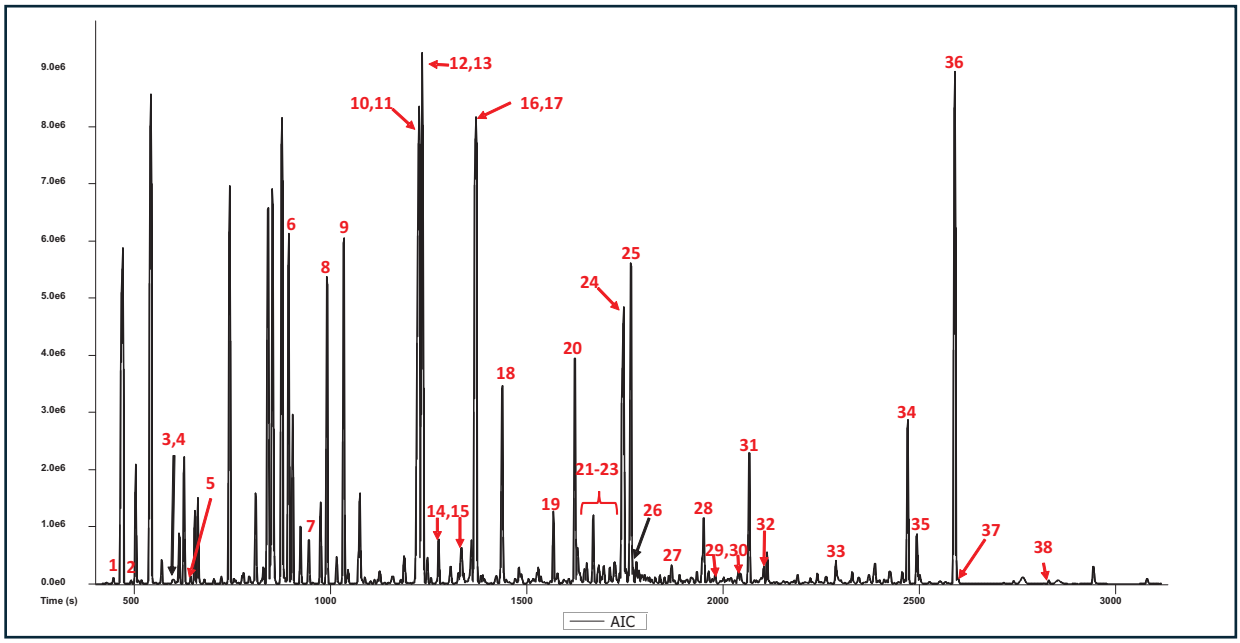


Figure 2. EI-HRT AIC of a Yeast Autolysate.

Table 2. Representative Compounds in Yeast Autolysate.

Peak #	Name	Formula	R.T. (s)	Area	Similarity
1	Pyruvic Acid (MEOX, TMS)	$C_7H_{15}NO_3Si$	446	1137387	934
2	Glycolic acid (2TMS)	$C_8H_{20}O_3Si_2$	491	715276	920
3	2-Furancarboxylic Acid (TMS)	$C_8H_{12}O_3Si$	588	236557	910
4	Oxalic Acid (2TMS)	$C_8H_{18}O_4Si_2$	598	982722	920
5	3-Hydroxybutyric Acid (2TMS)	$C_{10}H_{24}O_3Si_2$	643	1061101	900
6	Isoleucine(2TMS)	$C_{12}H_{29}NO_2Si_2$	876	80877728	964
7	Glyceric Acid (3TMS)	$C_{12}H_{30}O_4Si_3$	944	3040518	831
8	Serine (3TMS)	$C_{12}H_{31}NO_3Si_3$	990	37929232	925
9	Threonine (3TMS)	$C_{13}H_{33}NO_3Si_3$	1033	42516832	948
10	Methionine (2TMS)	$C_{11}H_{27}NO_2SSi_2$	1220	15297318	825
11	5-oxo-Proline (2TMS)	$C_{11}H_{23}NO_3Si_2$	1226	210548974	928
12	Aspartic Acid (3TMS)	$C_{13}H_{31}NO_4Si_3$	1233	73421702	957
13	4-Aminobutanoic acid (3TMS)	$C_{13}H_{33}NO_2Si_3$	1235	46969520	924
14	Cysteine (3TMS)	$C_{12}H_{31}NO_2SSi_3$	1275	5484621	963
15	Ornithine (3TMS)	$C_{14}H_{36}N_2O_2Si_3$	1358	5209095	908
16	Phenylalanine (2TMS)	$C_{15}H_{27}NO_2Si_2$	1367	52692227	955
17	Glutamic Acid (3TMS)	$C_{14}H_{33}NO_4Si_3$	1371	97699927	866
18	Asparagine (3TMS)	$C_{13}H_{32}N_2O_3Si_3$	1437	24267014	952
19	Glycerophosphate (4TMS)	$C_{15}H_{41}O_6PSi_4$	1567	5723912	905

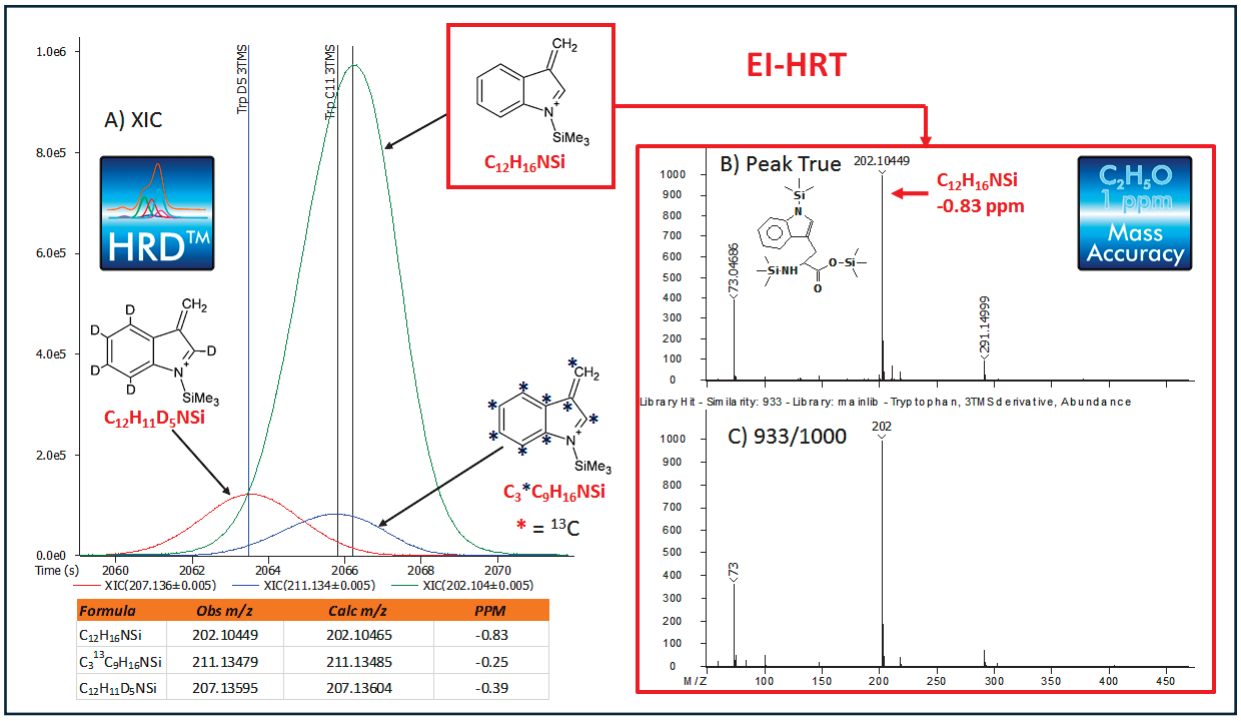
Peak #	Name	Formula	R.T. (s)	Area	Similarity
20	Ornithine (4TMS)	$C_{17}H_{44}N_2O_2Si_4$	1622	27497881	945
21	Mannose (5TMS)	$C_{21}H_{52}O_6Si_5$	1629	3629958	840
22	Adenine (2TMS)	$C_{11}H_{21}N_5Si_2$	1669	10309603	903
23	Cadaverine (4TMS)	$C_{17}H_{46}N_2Si_4$	1736	1858360	876
24	Lysine (4TMS)	$C_{18}H_{46}N_2O_2Si_4$	1746	35951466	835
25	Tyrosine (3TMS)	$C_{18}H_{35}NO_3Si_3$	1765	73102265	959
26	D-Sorbitol (6TMS)	$C_{24}H_{62}O_6Si_6$	1785	1068732	814
27	Xanthine (3TMS)	$C_{14}H_{28}N_4O_2Si_3$	1862	1355710	919
28	myo-Inositol (6TMS)	$C_{24}H_{60}O_6Si_6$	1950	5944599	912
29	Guanine (3TMS)	$C_{14}H_{29}N_5OSi_3$	1972	1391280	911
30	Spermine (6TMS)	$C_{28}H_{74}N_4Si_6$	2036	612262	907
31	Tryptophan (3TMS)	$C_{20}H_{36}N_2O_2Si_3$	2066	3042893C	927
32	Spermidine (5TMS)	$C_{22}H_{59}N_3Si_5$	2103	1388814	940
33	myo-Inositol Phosphate (7TMS)	$C_{27}H_{69}O_9PSi_7$	2287	2058184	943
34	Adenosine (4TMS)	$C_{22}H_{45}N_5O_4Si_4$	2470	14863948	953
35	Maltose (8TMS)	$C_{36}H_{86}O_{11}Si_8$	2493	6188006	939
36	Trehalose (8TMS)	$C_{36}H_{86}O_{11}Si_8$	2590	77375142	961
37	Guanosine (5TMS)	$C_{25}H_{53}N_5O_5Si_5$	2598	718577	804
38	5'-Adenosine Monophosphate (5TMS)	$C_{25}H_{54}N_5O_7PSi_5$	2830	454839	887

Accurate mass molecular and fragment ions were leveraged to confirm the identity of compounds. For example, observed m/z and calculated m/z values for molecular and fragment ions for a small set of amino acids are listed in Table 3. The average absolute mass accuracy in parts per million (ppm) for these amino acids was 0.72.

ChromaTOF data acquisition and data processing software combines the power of accurate mass and high resolving power for an extra dimension of separation—High Resolution Deconvolution (HRD). This is exemplified in Figure 3 where *ChromaTOF* processing resulted in deconvolution of coeluting native and labeled (D_5 and $^{13}C_{11}$) tryptophan (3TMS). The Peak True (Deconvoluted) mass spectrum was searched against the NIST 14 database and resulted in a match of 933/1000 for the native amino acid. Mass accuracy values for the D_5, $^{13}C_9$, and native $[M\text{-}C_8H_{20}NO_2Si_2]^+$ fragments were -0.39, -0.25, and -0.83 ppm respectively.

Table 3. Accurate Mass Values for Amino Acids in Yeast Autolysate.

Name	Formula	R.T. (s)	Ion	Obs m/z	Calc m/z	PPM
Glycine (3TMS)	$C_{11}H_{29}NO_2Si_3$	893	$M^{\bullet+}$	291.14974	291.15006	-1.09
Serine (3TMS)	$C_{12}H_{31}NO_3Si_3$	990	$[M\text{-}C_4H_9O_2]^+$	204.12358	204.12344	0.68
Methionine (2TMS)	$C_{11}H_{27}NO_2SSi_2$	1220	$M^{\bullet+}$	293.12956	293.12955	0.00
5-oxo-Proline (2TMS)	$C_{11}H_{23}NO_3Si_2$	1226	$M^{\bullet+}$	273.12096	273.1211	-0.50
Aspartic Acid (3TMS)	$C_{13}H_{31}NO_4Si_3$	1233	$M^{\bullet+}$	349.15586	349.15554	0.92
Ornithine (3TMS)	$C_{14}H_{36}N_2O_2Si_3$	1358	$M^{\bullet+}$	348.20799	348.20791	0.22
Phenylalanine (2TMS)	$C_{15}H_{27}NO_2Si_2$	1367	$[M\text{-}C_7H_7]^+$	218.10245	218.10271	-1.18
			$[M\text{-}C_4H_9O_2]^+$	192.12014	192.1203	-0.86
Glutamic Acid (3TMS)	$C_{14}H_{33}NO_4Si_3$	1371	$M^{\bullet+}$	363.17114	363.17119	-0.13
Asparagine (3TMS)	$C_{13}H_{32}N_2O_3Si_3$	1437	$M^{\bullet+}$	348.17121	348.17152	-0.89
Ornithine (4TMS)	$C_{17}H_{44}N_2O_2Si_4$	1622	$M^{\bullet+}$	420.24718	420.24744	-0.60
Lysine (4TMS)	$C_{18}H_{46}N_2O_2Si_4$	1746	$M^{\bullet+}$	434.26272	434.26309	-0.84
Tyrosine (3TMS)	$C_{18}H_{35}NO_3Si_3$	1765	$[M\text{-}C_{10}H_{15}OSi]^+$	218.10243	218.10271	-1.30
Tryptophan (3TMS)	$C_{20}H_{36}N_2O_2Si_3$	2066	$[M\text{-}C_8H_{20}NO_2Si_2]^+$	202.10449	202.10465	-0.83



Formula	Obs m/z	Calc m/z	PPM
$C_{12}H_{16}NSi$	202.10449	202.10465	-0.83
$C_3{}^{13}C_9H_{16}NSi$	211.13479	211.13485	-0.25
$C_{12}H_{11}D_5NSi$	207.13595	207.13604	-0.39

Figure 3. A) XIC showing $[M-C_8H_{20}NO_2Si_2]^+$ for D_5, $^{13}C_9$, and Labeled Tryptophan (3TMS) Fragments. B) Peak True Mass Spectrum for Native Tryptophan (3TMS). C) NIST Library Mass Spectrum—Tryptophan (3TMS).

Complementary CI-HRT data was also used to confirm the identity of yeast compounds. For example, the XICs for the protonated, high resolution molecular ions of D6 (m/z = 619.34560, R = 31,529) and native (m/z = 613.30806, R = 32,553) *myo*-inositol (6TMS) are displayed in Figure 4. Formulas and mass accuracy values for these adduct ions were $C_{24}H_{55}D_6O_6Si_6$ (0.18 ppm) and $C_{24}H_{61}O_6Si_6$ (0.38 ppm) respectively.

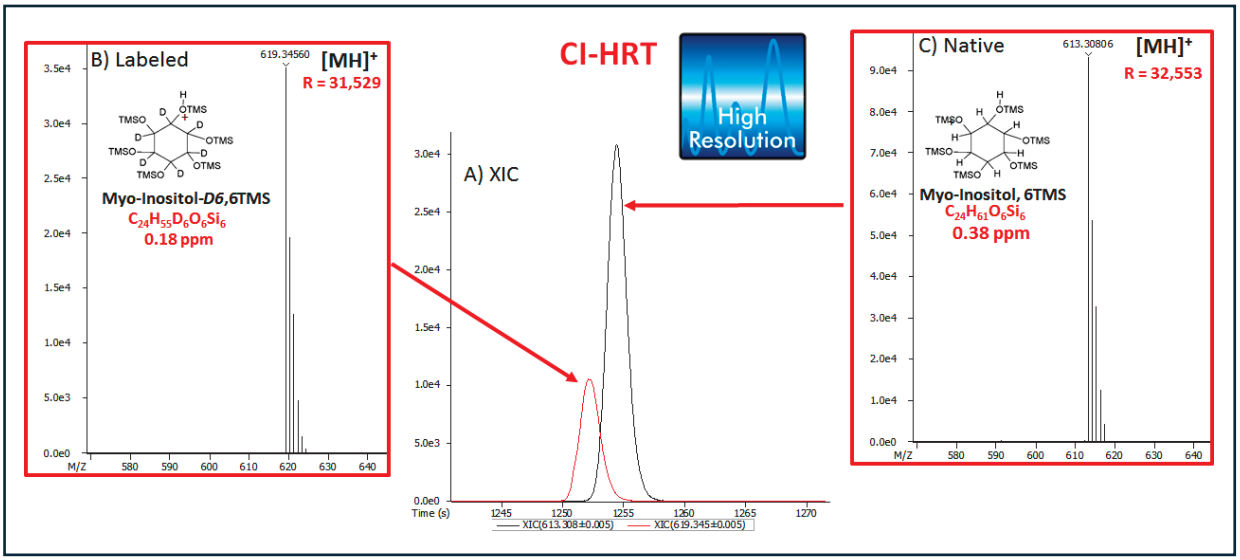


Figure 4. A) XIC of Labeled and Native Myo-Inositol. CI-HRT Peak True Mass Spectra for Labeled (B) and Native (C) Myo-Inositol.

The *Pegasus* GC-HRT 4D can be operated in 1D or 2D modes by a simple selection of operation modes in the software and specifying a modulation period. The enhanced chromatographic capability of the HRT 4D makes it an ideal discovery tool for metabolomics profiling as exemplified in the production of augmented mass spectral data with confidently identified compounds. An expansion of the contour plot for Baker's yeast extract shows the chromatographic resolution provided resulting from the GCxGC-HRT experiment (Figure 5). A comparison of the asparagine (Asn) spectral similarity results for data acquired in GC and GCxGC-HRT mode show a significant improvement in scores from 784 to 945/1000 (Figure 6). Additional examples illustrating the benefits of enhanced chromatographic resolution can be found in Table 4.

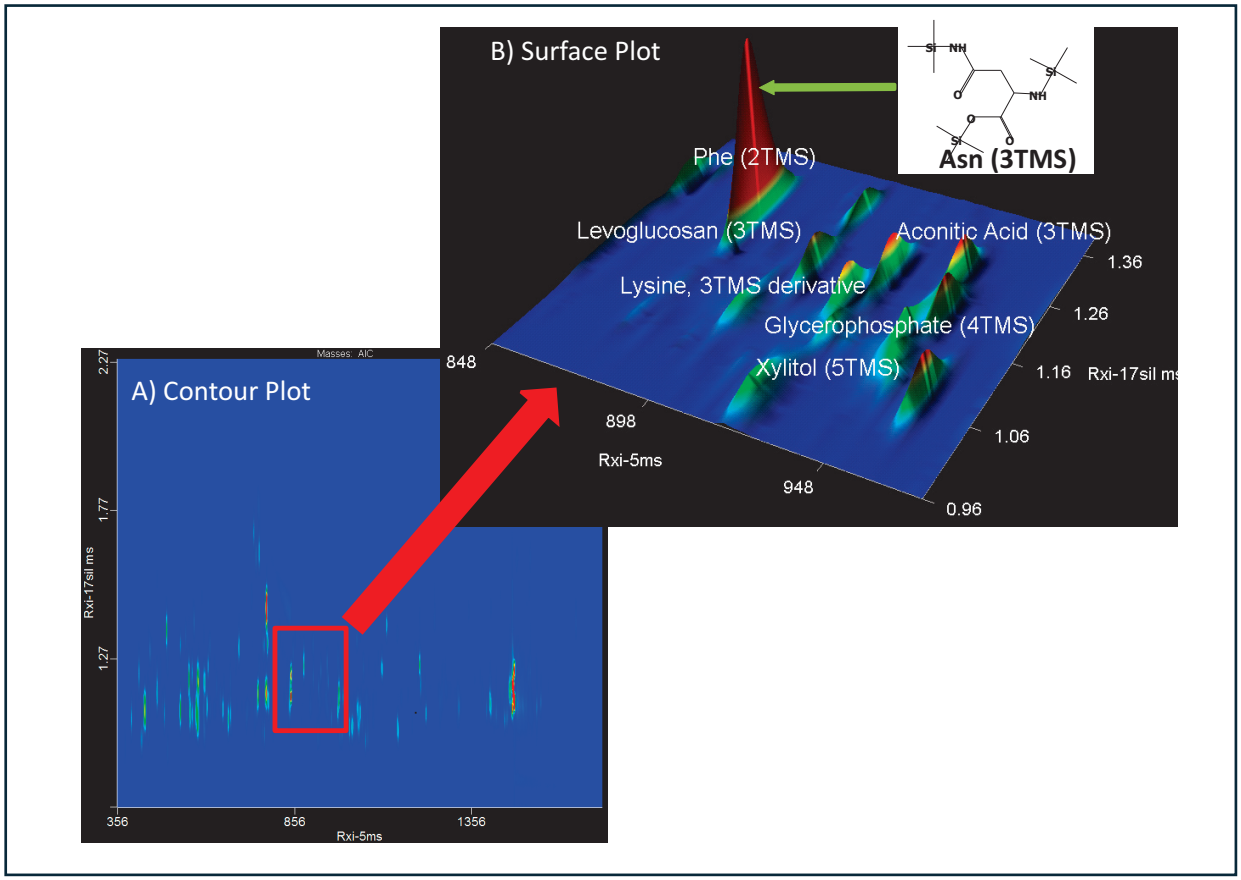


Figure 5. A) GCxGC-HRT Contour Plot for Baker's Yeast Extract. B) Expanded Surface Plot Region Showing Enhanced Chromatographic Resolution.

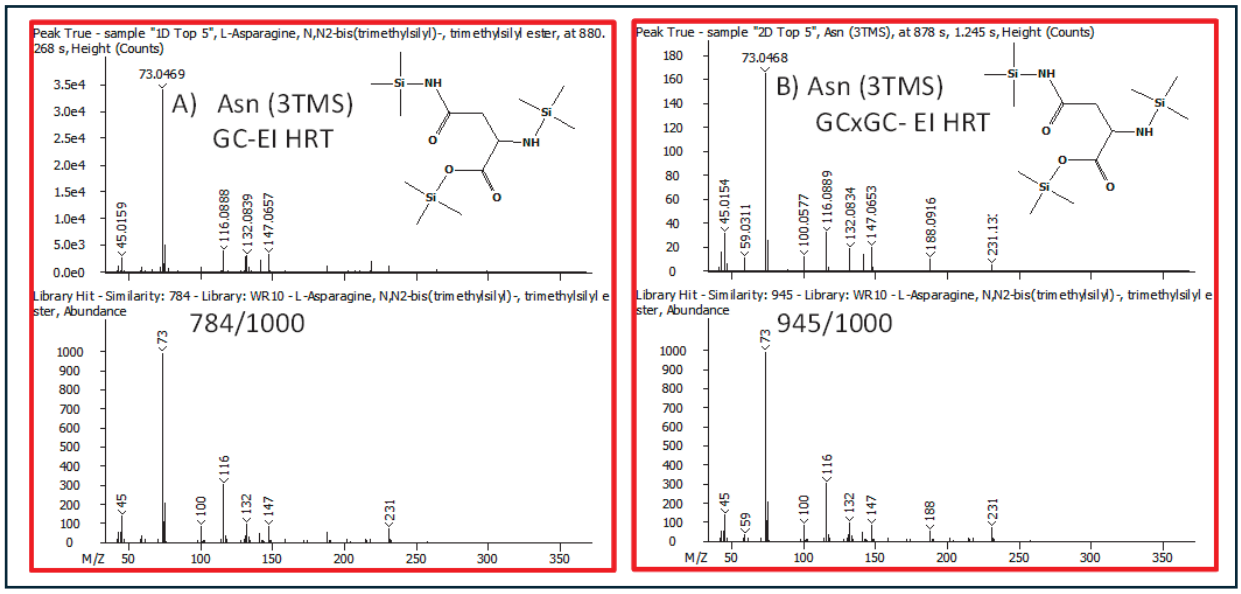


Figure 6. Comparison of GC-HRT Spectral Similarity (A) vs. GCxGC-HRT Similarity (B) for Asn (3TMS) in Baker's Yeast. Notice Improved score due to cleaner spectra resulting from enhanced separation power of GCxGC.

Table 4. A Comparison of Spectral Similarity Scores Obtained From GC versus GCxGC-HRT Data Acquisition of Baker's Yeast Extract. The cleaner spectra resulting from GCxGC have a significant effect on library similarity scores.

Name	Formula	Similarity (GC-HRT)	Similarity (GCxGC-HRT)
Phe (2TMS)	$C_{15}H_{27}NO_2Si_2$	**812**	**916**
2-Deoxyerythropentonic Acid (4TMS)	$C_{17}H_{42}O_5Si_4$	**645**	**870**
Asn (3TMS)	$C_{13}H_{32}N_2O_3Si_3$	**784**	**945**
Levoglucosan (3TMS)	$C_{15}H_{34}O_5Si_3$	**805**	**881**
Methyl tetradecanoate	$C_{15}H_{30}O_2$	**933**	**942**
Xylitol (5TMS)	$C_{20}H_{52}O_5Si_5$	**857**	**890**
(E)-Aconitic Acid (3TMS)	$C_{15}H_{30}O_6Si_3$	**768**	**910**
Glutamine (3TMS)	$C_{14}H_{34}N_2O_3Si_3$	**835**	**923**
Glycerophosphate (4TMS)	$C_{15}H_{41}O_6PSi_4$	**735**	**879**

Conclusion

LECO's *Pegasus* GC-HRT 4D and *ChromaTOF* brand software are powerful tools for confident analysis of metabolomics samples. The combination of EI and CI-HRT high resolution, accurate mass data facilitates rapid compound identification. This was illustrated by Peak Find processing and its ability to separate and identify coeluting deuterated, ^{13}C labeled and native compounds in complex yeast matrices. The addition of an extra dimension of chromatographic resolution dramatically enhances the discovery capabilities of the GC-HRT 4D making it an extremely valuable instrument for metabolite discovery workflows.

References

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