Effective Comparison of Yeast Extracts Using High Resolution GC and GC×GC-HRTOFMS

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Introduction

Yeast has been used for centuries in the production of beer, wine, and baked goods.[1] In modern times, yeast has become a "cellular factory" for the manufacture of bioethanol, lactic acid, and other valuable organic molecules.[2-4] In order for this bioengineering to become realized, the metabolism of yeast must be clearly understood. Unfortunately, yeast is difficult to study due to the chemical and physical complexity of metabolites and their wide concentration range. Furthermore, the bottleneck in metabolomics research and engineering is the characterization of the diverse features present in these samples. In this study we explored the utilization of gas chromatography high resolution time-of-flight mass spectrometry (GC-HRT) and GC×GC-HRT for efficient metabolite chromatographic separation, minimization of background interference, resolution of isobaric interferences, and rapid compound identification.

Methods

Objectives

1) Demonstrate the utility of multidimensional chromatography and high resolution mass spectrometry for rapid and confident identification of yeast metabolites.

Fig. 1: LECO Pegasus® GC-HRT 4D.

- *Speed and robustness*
- *Unprecedented chromatographic resolution*
- *EI and CI data acquisition*
- *High resolution deconvolution*
- *High quality spectral data for spectral similarity searches (NIST, Wiley, etc.)*
- *High resolution accurate mass measurements for fragment, molecular, and adduct ion formula determinations*

2) Investigate the effectiveness of yeast extraction using a mixed solvent system ($H_2O/CH_3OH/CHCl_3$).
3) Compare metabolites partitioned between polar and non-polar phases.
4) Pinpoint key metabolites differentiating commercial dry yeast samples via statistical analysis.[5]

Samples

- 2 yeast autolysate samples (A and B); GC and GC×GC system capabilities
- Dry baker's yeast samples (1–5); Extraction investigations

Extraction

Yeast (15 to 30 mg) was extracted with 5 mL 0.5:0.5:1 $H_2O/CH_3OH/CHCl_3$ and filtered into 2 mL GC vials. The solvents were evaporated using a Speed Vac and residual water was removed with a lyophilizer.

Derivatization

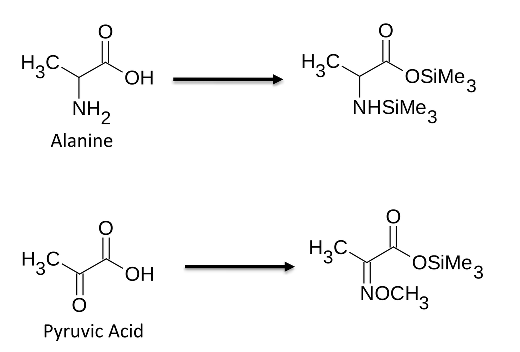

Fig. 2: Derivatization of Alanine and Pyruvic Acid.

a) 20 µL of MEOX (20 mg/mL methoxyamine in pyridine), 1 hour at 60°C
b) 80 µL of MSTFA, 1 hour at 60°C

Instrument Parameters

Gas Chromatograph	Agilent 7890 with MPS2 Autosampler
Injection	1 µL, split 20:1 @ 250°C
Carrier Gas	He @ 1.0 ml/min, Constant Flow
Column	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating
Oven Program	1 min at 60°C, ramped 10°C/min to 320°C, held 10 min
Transfer Line	300°C
Mass Spectrometer	**LECO Pegasus HT**
Ion Source Temperature	250°C (CI: 200°C)
Mass Range	35-510 m/z (CI 60 -1200)
Acquisition Rate	10 spectra/s

Gas Chromatograph	Agilent 7890 with Dual Stage Quad Jet Modulator and MPS2 Autosampler
Injection	1 µL, split 20:1 @ 250°C
Carrier Gas	He @ 1.0 ml/min, Corrected Constant Flow
Column One	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating
Column Two	Rxi-17SilMS, 2.0 m x 0.25 mm x 0.25 µm coating
Temperature Program	1 min at 60°C, ramped 10°C/min to 320°C, held 10 min Secondary oven maintained +5°C relative to primary oven
Modulation	3 s with temperature maintained +15°C relative to 2nd oven
Transfer Line	300°C
Mass Spectrometer	**LECO Pegasus HRT**
Ion Source Temperature	250°C (CI: 200°C)
Mass Range	35-510 m/z (CI 60 -1200)
Acquisition Rate	200 spectra/s

Results: GC-HRT (Yeast A)

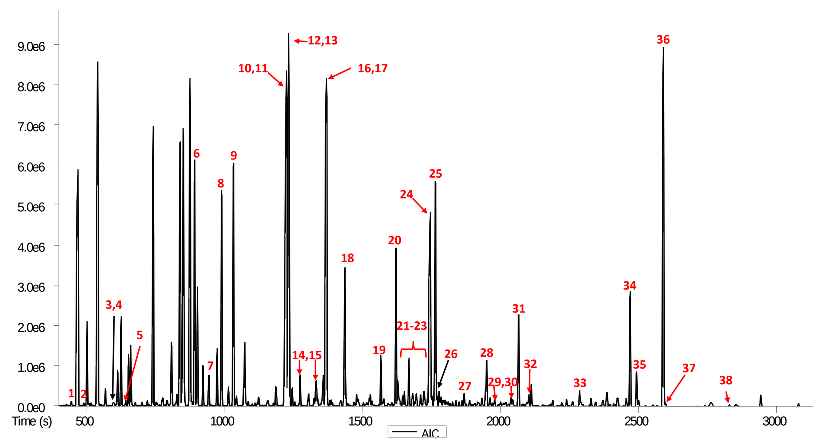

Fig. 3: Analytical Ion Chromatogram (AIC) Yeast Extract A.

Table 1: Representative Compounds in Yeast Extract A.

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
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	30428930	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

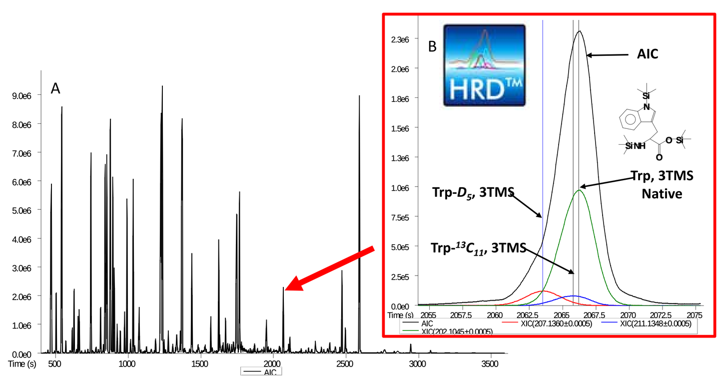

Fig. 4: AIC and Extracted Ion Chromatogram (XIC) For D_5, $^{13}C_{11}$ and Native Trp (3TMS).

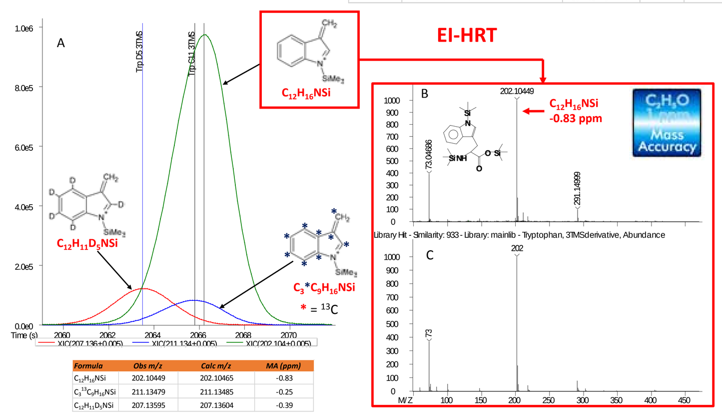

Fig. 5: A) Mass Accuracy Values for D_5, $^{13}C_{11}$ and Native Trp (3TMS). B) Peak True and C) Library Mass Spectra Native Trp (3TMS).

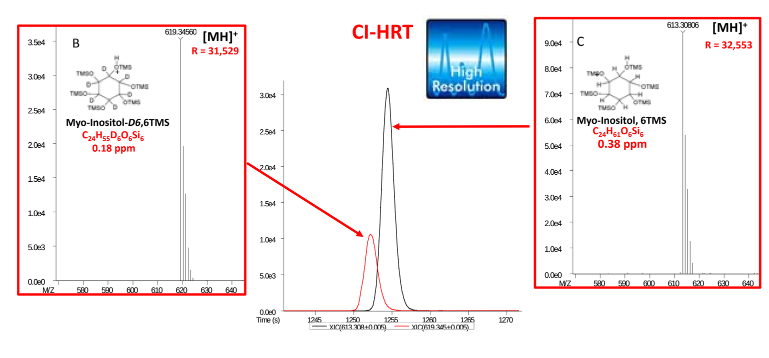

Fig. 6: A) XIC for D6 and Native myo-Inositol. CI-HRT Mass Spectra for B) D6, and C) Native myo-Inositol.

Results: GC×GC-HRT (Yeast B)

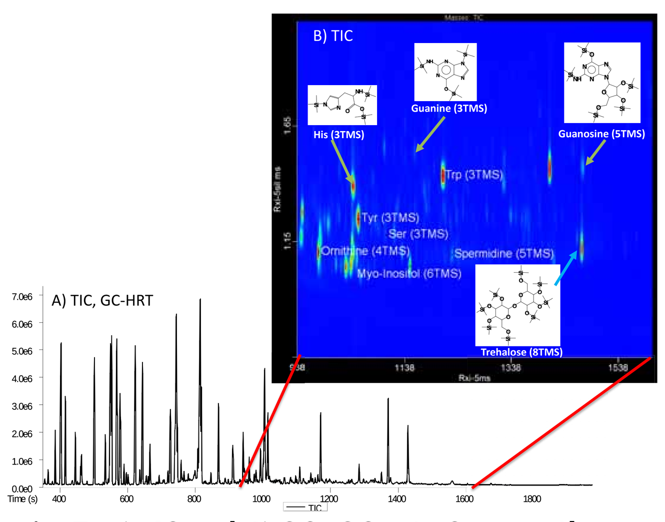

Fig. 7: A) TIC and B) GC×GC-HRT Contour Plot for Yeast Extract B.

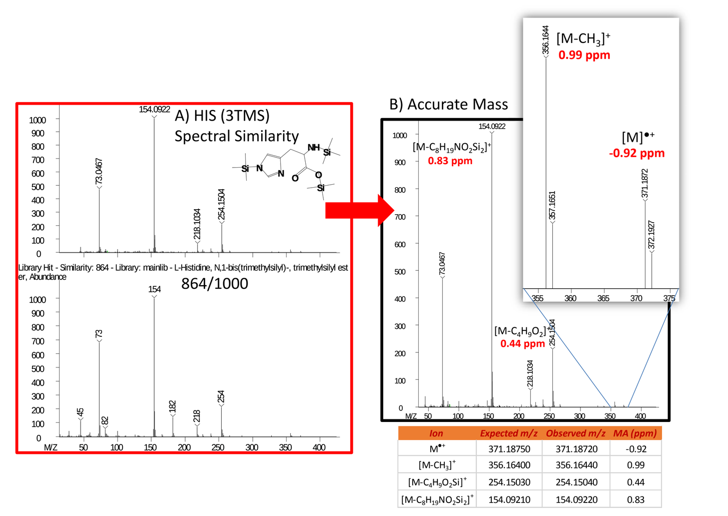

Fig. 8: A) Peak True and Library Mass Spectra for His (3TMS). B) Expansion of Peak True Mass Spectrum and Mass Accuracy Values for His (3TMS).

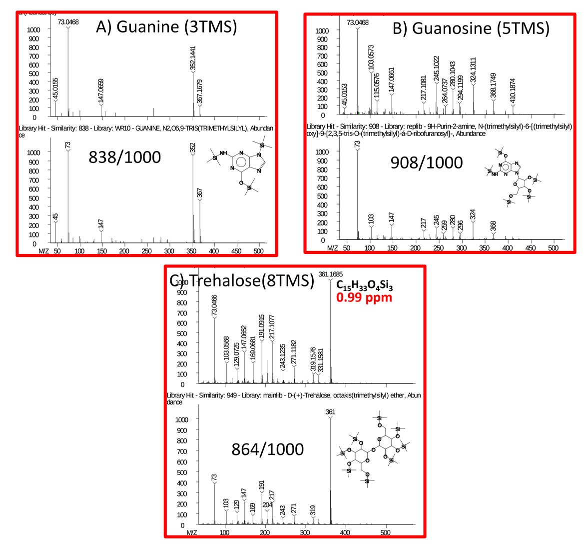

Fig. 9: Peak True and Library Mass Spectra for A) Guanine (3TMS), B) Guanosine (5TMS), and C) Trehalose (8TMS).

Results: GC-HRT Comparison of Dry Yeast Extracts 1–5

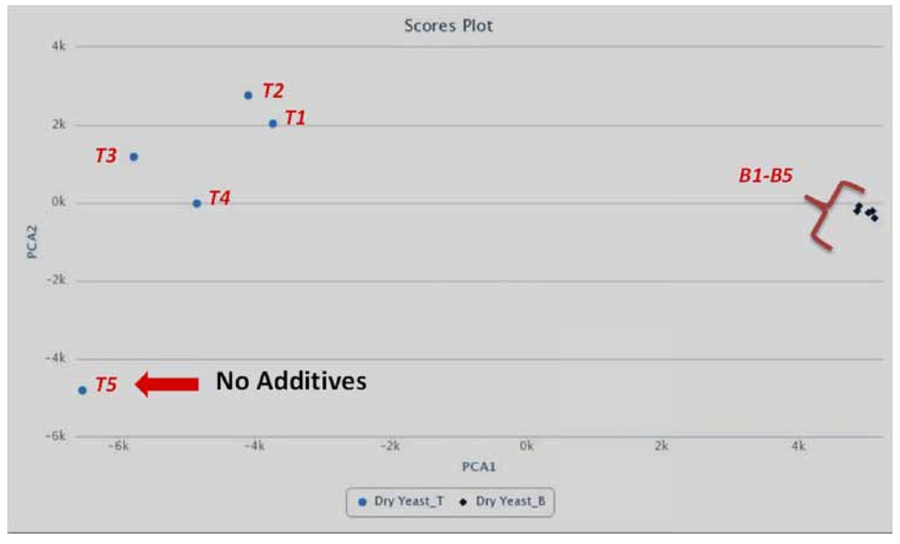

Fig. 10: PCA Plot (Fold Change > 1.5, P < 0.05) for Baker's Yeast Samples Extracted Using a Two Phase Solvent System (T = Aqueous, B = Organic). Sample 5 Did Not Contain Additional Additives (e.g., ascorbic acid, sorbitan monostearate).

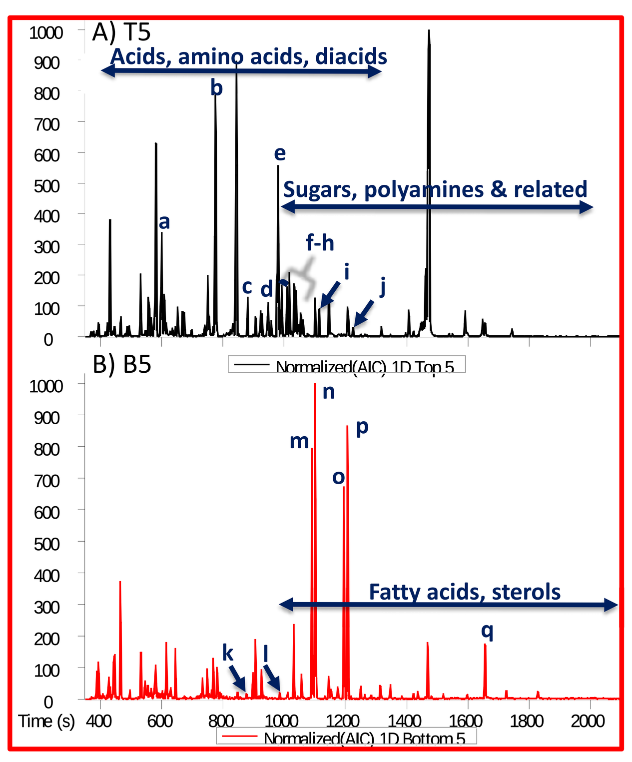

Fig. 11: Top (Aqueous) and Bottom (Organic) AICs for Yeast Extract 50

Tables 2 and 3: Yeast Extract 5 Representative Compounds Extracted Into Top (Aqueous) and Bottom (Organic) Phases.

Top (Aqueous)

Peak	Name	R.T. (s)	Formula	Area	Similarity
a	Proline (2TMS)	600	$C_{11}H_{25}NO_2Si_2$	14228968	925
b	Glutamic Acid (3TMS)	844	$C_{14}H_{33}NO_4Si_3$	19581921	918
c	Asparagine (3TMS)	880	$C_{13}H_{32}N_2O_3Si_3$	3150080	784
d	Glutamine (3TMS)	946	$C_{14}H_{34}N_2O_3Si_3$	939026	835
e	Citric Acid (4TMS)	980	$C_{18}H_{40}O_7Si_4$	10913589	839
f	Fructose (MEOX, 5TMS)	1009	$C_{22}H_{55}NO_6Si_5$	3549185	815
g	Mannose (MeOX, 5TMS)	1035	$C_{22}H_{55}NO_6Si_5$	3311292	933
h	Galactose (MEOX, 5TMS)	1046	$C_{22}H_{55}NO_6Si_5$	958196	919
i	Cys-Gly (3Tms)	1114	$C_{14}H_{34}N_2O_3SSi_3$	1755190	924
j	Spermidine (5TMS)	1225	$C_{22}H_{59}N_3Si_5$	610033	875

Bottom (Organic)

Peak	Name	R.T. (s)	Formula	Area	Similarity
k	Lauric Acid (TMS)	859	$C_{15}H_{32}O_2Si$	21301	768
l	Myristic Acid (TMS)	985	$C_{17}H_{36}O_2Si$	42326	771
m	Palmitoleic Acid (TMS)	1090	$C_{19}H_{38}O_2Si$	1100651	920
n	Palmitic Acid (TMS)*	1101	$C_{19}H_{40}O_2Si$	2764956	916
o	(*E*)-Oleic Acid (TMS)	1195	$C_{21}H_{42}O_2Si$	865860	924
p	Stearic Acid (TMS)*	1207	$C_{21}H_{44}O_2Si$	1925246	898
q	Ergosterol**	1656	$C_{31}H_{52}OSi$	250513	849

Results: GC and GC×GC-HRT Dry Yeast Extract #5

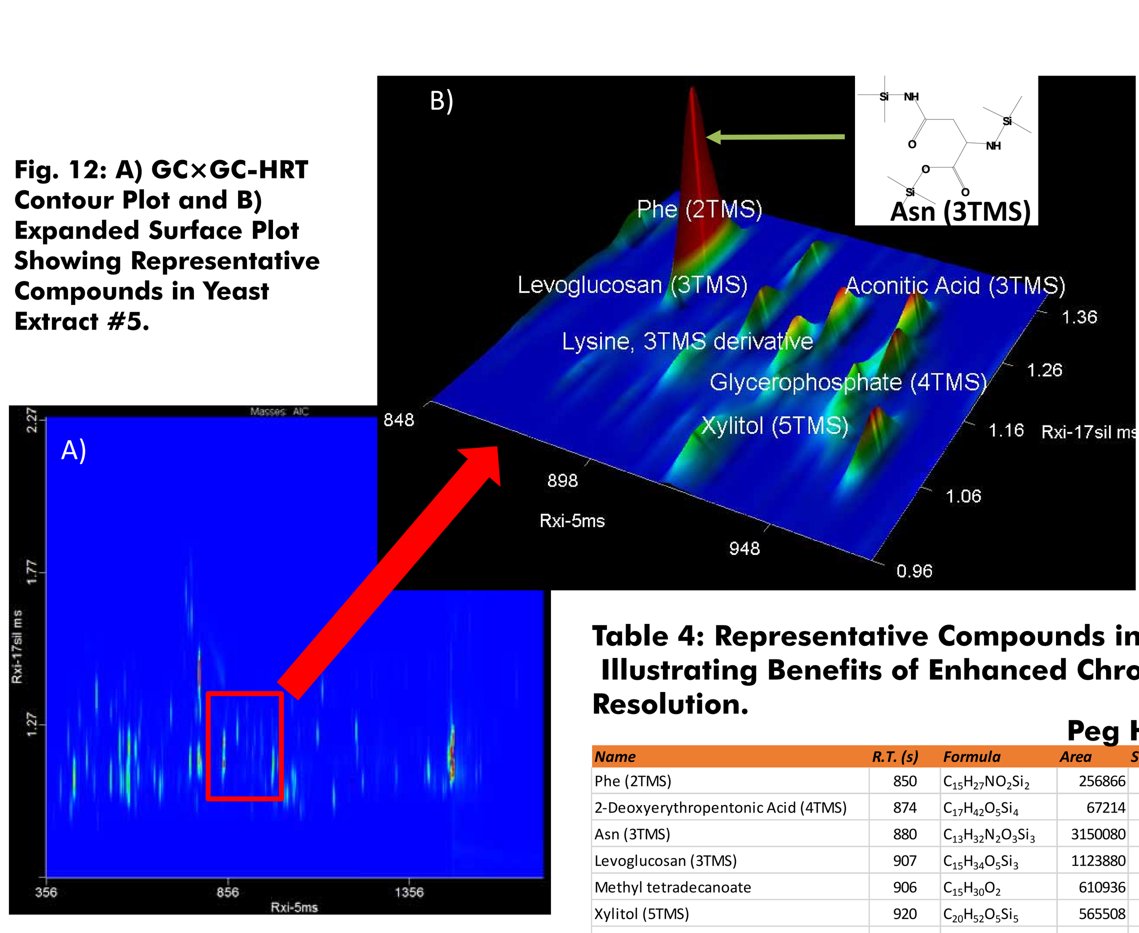

Fig. 12: A) GC×GC-HRT Contour Plot and B) Expanded Surface Plot Showing Representative Compounds in Yeast Extract #5.

Table 4: Representative Compounds in Yeast 6 Extract Illustrating Benefits of Enhanced Chromatographic Resolution.

Name	R.T. (s)	Formula	Area	Similarity	Peg HRT → Peg HRT 4D R.T. (s)	Peg HRT → Peg HRT 4D Similarity
Phe (2TMS)	850	$C_{15}H_{27}NO_2Si_2$	256866	812	851, 1.29	916
2-Deoxyerythropentonic Acid (4TMS)	874	$C_{17}H_{42}O_5Si_4$	67214	645	875, 1.06	870
Asn (3TMS)	880	$C_{13}H_{32}N_2O_3Si_3$	3150080	784	881, 1.24	945
Levoglucosan (3TMS)	907	$C_{15}H_{34}O_5Si_3$	1123880	805	908, 1.21	881
Methyl tetradecanoate	906	$C_{15}H_{30}O_2$	610936	933	908, 1.32	942
Xylitol (5TMS)	920	$C_{20}H_{52}O_5Si_5$	565508	857	920, 1.01	890
(E)-Aconitic Acid (3TMS)	928	$C_{15}H_{30}O_6Si_3$	1155386	768	929, 1.23	910
Glutamine (3TMS)	946	$C_{14}H_{34}N_2O_3Si_3$	939026	835	947, 1.25	923
Glycerophosphate (4TMS)	944	$C_{15}H_{41}O_6PSi_4$	250503	735	944, 1.15	879

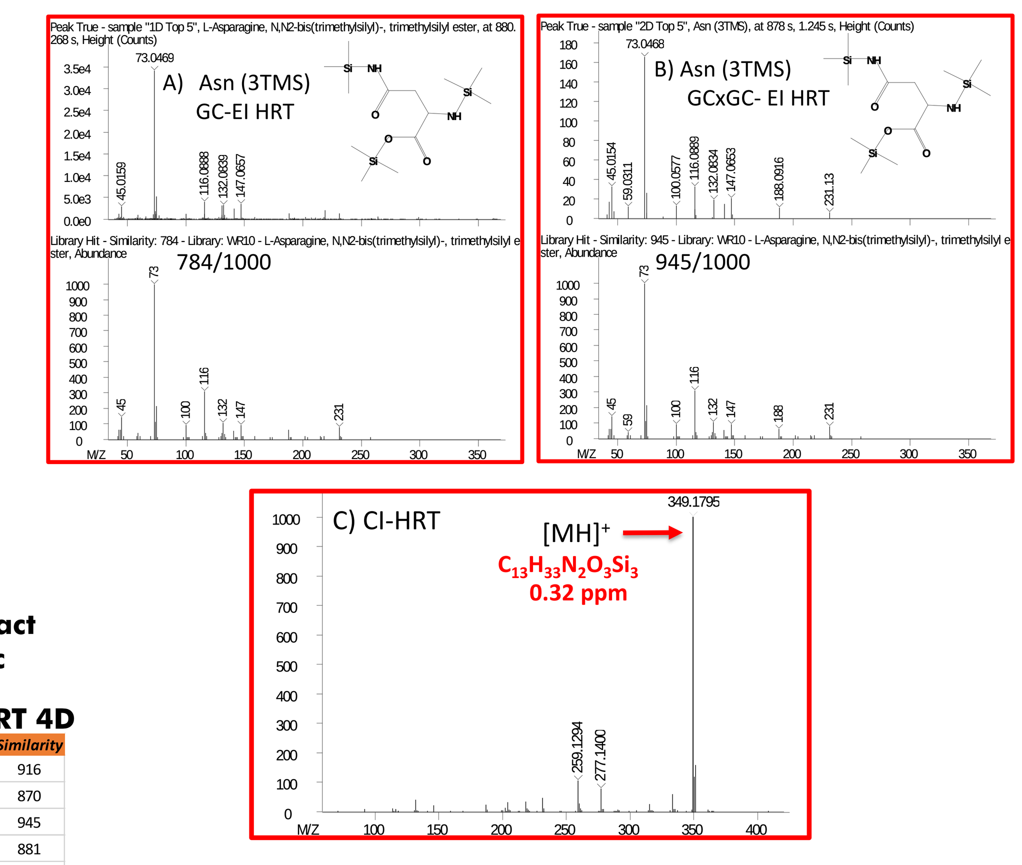

Fig. 13: Peak True and Library Mass Spectral Data for Asn (3TMS) Acquired Using A) *Pegasus* GC-HRT and B) *Pegasus* GC-HRT 4D instruments. C) CI-HRT data for Asn (3TMS).

Conclusions

The *Pegasus* GC-HRT and GC-HRT 4D instruments are effective tools for the comprehensive analysis of yeast extracts.

- Enhanced chromatographic resolution of the HRT 4D system facilitated compound characterization by improving spectral similarity scores
- High quality data resulted in excellent spectral similarity scores when compared to large, well-established databases (NIST, Wiley)
- High resolution, accurate mass data was crucial for determination of fragment, molecular, and adduct formulas

A mixed solvent system was used for extraction of yeast metabolites.

- Polar protic solvents such as methanol and water are effective solvents for extraction of small polar and/or ionic metabolites
- Less polar solvents (e.g., chloroform) are superior for the extraction of fatty acids, sterols, and other relatively non-polar materials

References

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