Comparison of GC-MS and GC×GC-MS in the Analysis of Human Serum Samples for Biomarker Discovery

Jason H. Winnike[⊥¶], Xiaoli Wei[†¶], Kevin J. Knagge[⊥], Steven D. Colman[⊥], Simon G. Gregory[⊥‡], Xiang Zhang[†*]

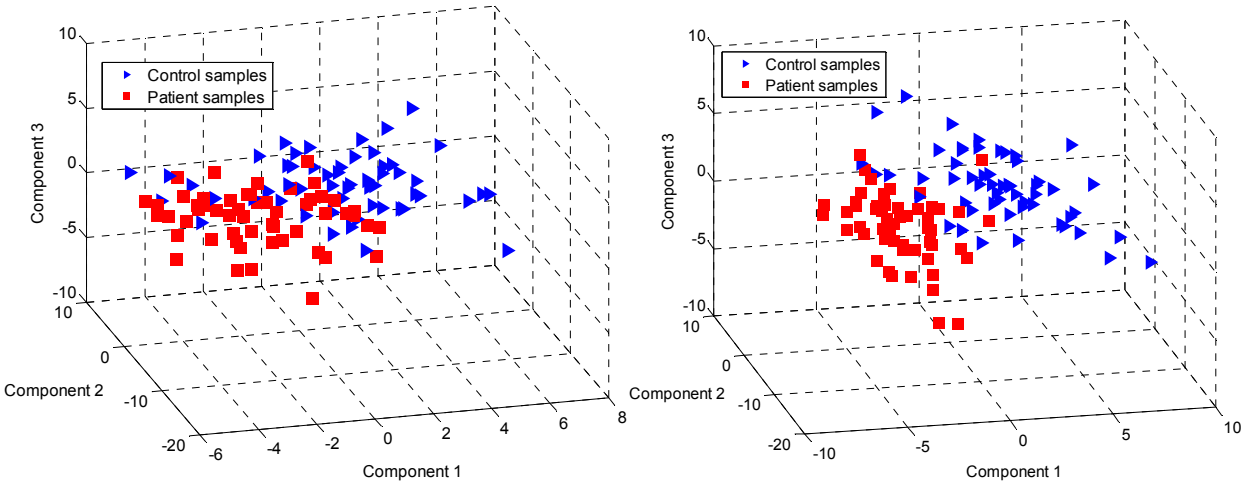
[⊥] David H. Murdock Research Institute, Kannapolis, NC 28081, USA.

[†] Department of Chemistry, Center of Regulatory & Environmental Analytical Metabolomics (CREAM), University of Louisville, KY 40292, USA.

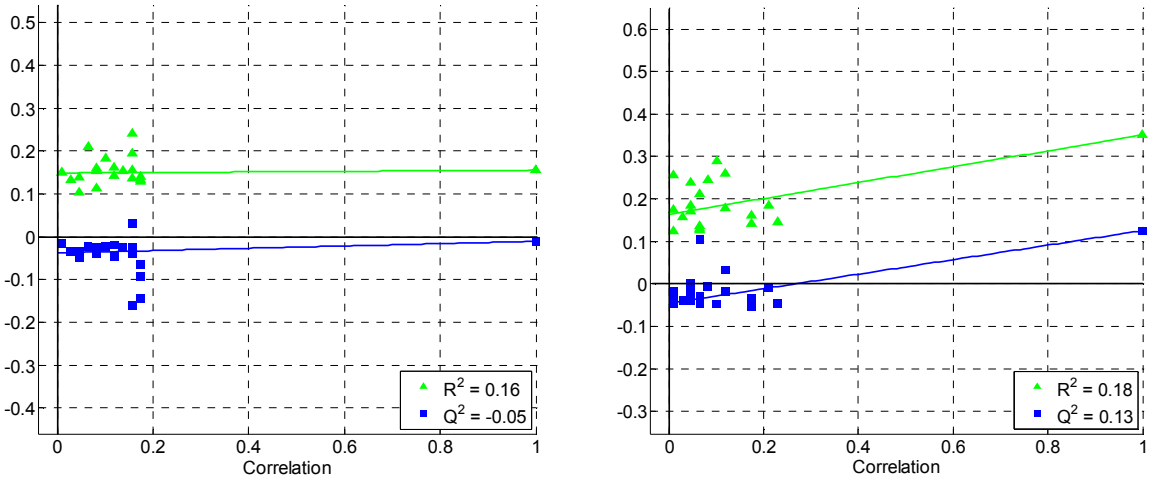
[‡] Duke Molecular Physiology Institute, Department of Medicine, Duke University, Durham, NC 27701, USA.

[¶] These two authors contributed equally to this study.

CORRESPONDING AUTHOR: Xiang Zhang, Ph.D. Department of Chemistry, University of Louisville, 2320 South Brook Street, Louisville, KY 40292, USA. Phone: +01 502 852 8878. Fax: +01 502 852 8149. E-mail: xiang.zhang@louisville.edu

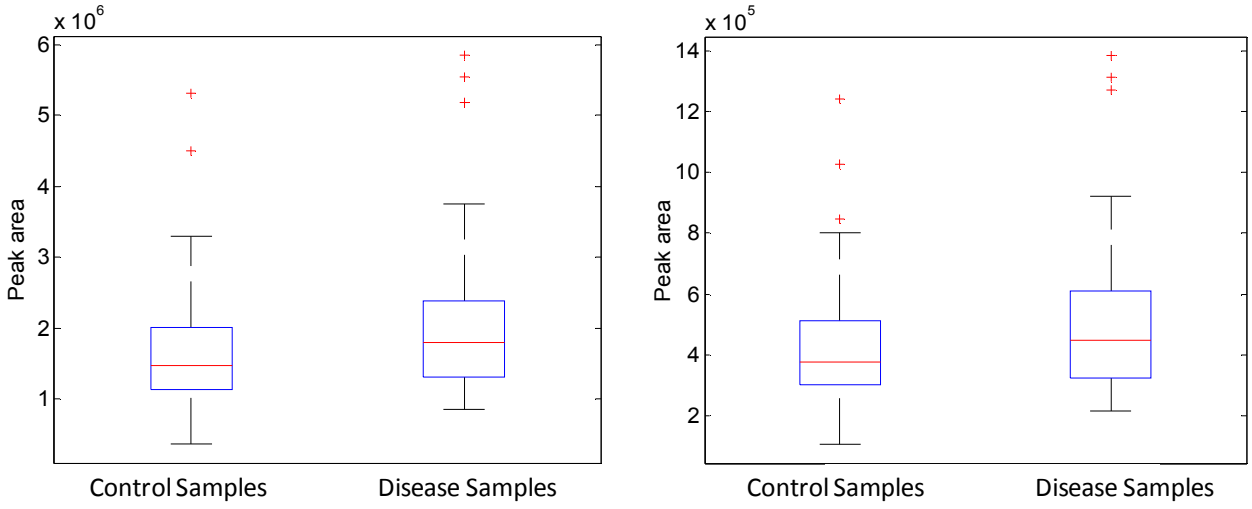


(A) **(B)**



(C) **(D)**

Figure S1. PLSDA analysis of the metabolite profiles of all patient samples. (A) is the clustering result of GC-MS data. (B) is the clustering result of GC×GC-MS data. (C) is the cross validation result of the PLSDA for analysis of GC-MS data. The magnitudes of R^2 and Q^2 are (R^2 = 0.16, Q^2 = -0.05). (D) is the cross validation results of the PLSDA for analysis of GC×GC-MS data with R^2 = 0.18 and Q^2 = 0.13.



(A) (B)

Figure S2. Abundance distribution of metabolite 5 between the control and the patient samples. The fold-change of this metabolite between the patient samples and the control samples is 1.18 with $p = 0.018$ in the GC-MS data, and 1.17 in the GC×GC-MS data with $p = 0.022$.

Table S1. Data processing parameters used in LECO ChromaTOF.

Parameters Applicable to Both GC-MS and GC×GC-MS	
Baseline Offset	1
Number of Data Points Averaged for Baseline Smoothing	Auto
Peak Width for Peak Finding	GC-MS: 4, GC×GC-MS: 7.5
Minimum Number of Apexing Masses for Peak Finding	2
Minimum SNR for Peak Finding	50
Library Search Mode	Normal, Forward
Library Hits Returned per Peak	10
Minimum/Maximum Molecular Weight Allowed	0/1000
Relative Mass Threshold for Library Searching	10
Minimum Similarity Match Before Name is Assigned	600
Libraries Used for Searching	In-House, LECO-Fiehn Rtx5, NIST
Mass Used for Area/Height Calculation	Unique
Parameters Specific to GC×GC-MS	
Match Required to Combine Peaks in the Second Dimension	500
Second Dimension Peak Width	0.15
Minimum SNR for Subpeak to be Retained	6
Integration Approach	Traditional

Table S2. Metabolite peaks detected by the GC-MS platform as potential biomarkers that have significant abundance differences between the patient and control samples. Fold change is the ratio of mean peak area of a metabolite in the patient samples divided by the mean peak area of the same metabolite in the control samples.

Peak ID	$^{1}t_R$ (s)	*p* value	Fold change
1	2361	0.009	1.31
2	1124	0.020	0.87
3	2087	0.004	0.49
4	1947	0.017	1.23
5	2394	0.018	1.18
6	2167	0.001	1.40
7	1779	0.001	0.90
8	2033	0.006	1.15
9	2352	0.008	0.74
10	1346	0.004	0.80
11	736	0.007	0.87
12	1462	0.037	0.90
13	956	0.034	1.48
14	1648	0.046	0.91
15	936	0.004	0.86
16	886	0.015	0.91
17	2176	0.035	0.91
18	2349	0.036	0.87
19	2626	0.036	1.10
20	2368	0.000	1.46
21	1671	0.012	0.89
22	1692	0.025	1.08
23	1082	0.024	1.08

Table S3. Metabolite peaks detected by the GC×GC-MS platform as potential biomarkers that have significant abundance differences between the patient and control samples. Fold change is the ratio of mean peak area of a metabolite in the patient samples divided by the mean peak area of the same metabolite in the control samples.

Peak ID	${}^{1}t_R$ (s)	${}^{2}t_R$ (s)	*p* value	Fold change
1	2372	1.336	0.024	1.25
2	1135	1.041	0.008	0.86
3	2093	1.038	0.021	0.66
4	1958	1.120	0.003	1.31
5	2404	1.263	0.022	1.17
6	2173	1.236	0.001	1.37
7	1793	1.212	0.001	0.90
8	2046	1.402	0.000	1.21
9	2365	1.282	0.017	0.80
10	2322	1.139	0.031	1.11
11	1542	1.115	0.034	1.36
12	2108	1.040	0.006	0.47
13	2098	1.034	0.032	0.51
14	2130	1.024	0.000	3.03
15	1496	1.056	0.003	0.78
16	1501	1.066	0.003	0.78
17	1271	1.044	0.002	2.05
18	1145	1.035	0.014	0.92
19	2143	1.054	0.000	0.58
20	1237	1.002	0.031	0.93
21	1563	1.172	0.012	0.75
22	2412	1.188	0.006	0.87
23	922	1.120	0.013	0.89
24	998	1.178	0.032	1.36
25	2329	1.283	0.010	1.16
26	832	0.962	0.009	0.62
27	1363	1.365	0.007	1.20
28	2453	1.127	0.015	1.07
29	1904	1.342	0.000	0.62
30	1825	1.130	0.018	1.35
31	1751	1.356	0.002	1.16
32	1807	1.246	0.001	0.73
33	1850	1.086	0.011	1.13
34	2230	1.357	0.006	1.18