Electronic Supplementary Material (ESI) for Analyst.

Integrating Comprehensive Two-dimensional Gas Chromatography Mass Spectrometry and Parallel Two-dimensional Liquid Chromatography Mass Spectrometry for Untargeted Metabolomics

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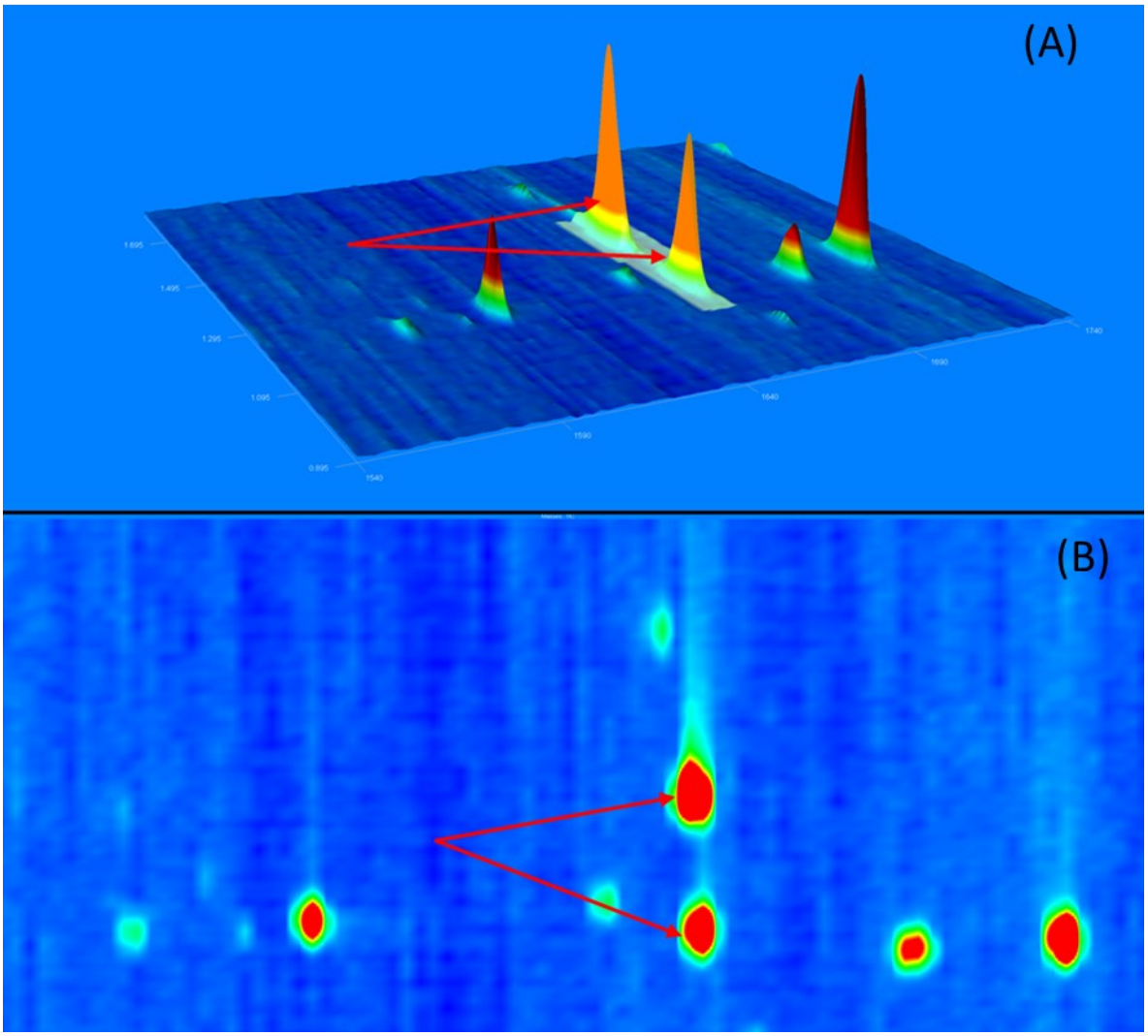

Figure S1. Two compounds co-eluted from the first dimension GC but separated by the second dimension GC. (A) is a three-dimensional view, and (B) is a contour plot.

Table S1. Data processing parameters used in LECO ChromaTOF software

Data processing parameters used in LECO ChromaTOF.	
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	10
Library Search Mode	Normal, Forward
Library Hits Returned per Peak	10
Minimum/Maximum Molecular Weight Allowed	29/850
Relative Mass Threshold for Library Searching	10
Minimum Similarity Match Before Name is Assigned	600
Libraries Used for Searching	In-House, NIST-14
Mass Used for Area/Height Calculation	Unique
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. Details of metabolites identified by MS/MS matching in 2DLC-MS (-) mode

Name	*m/z*	Retention time (min)	Database
(±)13-HODE	295.2273	13.96	In-house DB[a]
(R)-2-Hydroxybutyric acid	103.0399	2.13	In-house DB
2-Hydroxycaproic acid	131.0712	2.45	CD[b]
2-Methylglutaric acid	145.0505	2.17	CD
2-Oxobutyric acid	101.0242	5.54	CD
2-Oxoglutaric acid	145.0141	5.36	CD
2-Phosphoglycerate (2PG)	184.9854	0.88	In-house DB
3-AMP	346.0552	1.20	In-house DB
3-Hydroxybutyric acid	103.0400	1.68	In-house DB
3-Phosphoglyceric acid	184.9854	6.30	CD
4-Dodecylbenzenesulfonic acid	325.1837	4.52	CD
4-Methyl-2-oxovaleric acid	129.0556	7.53	In-house DB
4-Oxoproline	128.0352	4.53	CD
5-CMP	322.0443	0.94	In-house DB
Alanine	88.0402	0.83	In-house DB
Alpha-D-Glucose 1-phosphate	259.0234	0.83	In-house DB
alpha ketoglutaric acid	145.0141	1.15	In-house DB
Arabitol	151.0610	5.16	CD
Asparagine	131.0461	0.83	In-house DB
Aspartic Acid	132.0301	0.83	In-house DB
Azelaic acid	187.0974	9.05	In-house DB
Benzoic acid	121.0294	0.10	CD
Citrate	191.0196	1.21	In-house DB
Citrulline	174.0882	0.85	In-house DB
Cystathionine	221.0598	0.80	In-house DB
D-Erythrose-4-phosphate	199.0011	0.88	In-house DB
D-Glucosamine-6-phosphate	258.0381	5.97	In-house DB
D-Glucose (Glc)	179.0559	0.88	In-house DB
D-Glucose 6-phosphate	259.0218	6.20	CD
D-Glyceraldehyde 3-Phosphate (GADP)	168.9905	0.91	In-house DB
Dihydroxyacetone Phosphate (DHAP)	168.9905	4.96	In-house DB
DL-alpha-Glycerol phosphate	171.0062	0.86	In-house DB
DL-β-Leucine	130.0872	4.96	CD
Dodecanedioic acid	229.1442	9.59	CD
D-Ribulose-5-phosphate	229.0116	0.85	In-house DB

D-Saccharic acid	209.0300	0.86	CD
Esculin	339.0735	0.89	CD
Ethyl-β-D-glucuronide	221.0665	1.46	CD
Fructose	179.0559	5.56	CD
Fumaric acid	115.0036	1.53	In-house DB
Glutamic acid	146.0457	0.85	In-house DB
Glutamine	145.0617	5.74	CD
Glutaric acid	131.0349	2.44	In-house DB
Glutathione	306.0761	1.15	In-house DB
Glycerol 3-phosphate	171.0062	5.75	CD
Glycine	74.0246	0.83	In-house DB
Glycyl-L-leucine	187.1086	5.25	CD
Histidine	154.0620	0.79	In-house DB
Hypotaurine	108.0123	5.70	CD
Hypoxanthine	135.0311	1.18	CD
Inosine	267.0732	1.60	In-house DB
Isobutyric acid	87.0450	2.45	In-house DB
Isocitric acid	191.0195	0.89	In-house DB
Isoleucine	130.0872	1.75	In-house DB
Itaconic acid	129.0192	6.16	CD
Lactic acid	89.0243	1.24	In-house DB
L-alpha-Aminoadipic acid	160.0614	0.90	In-house DB
L-alpha-Amino-n-butyric acid	102.0559	0.85	In-house DB
L-Ascorbic acid 2-sulfate	254.9813	0.97	CD
Leukotriene B4	335.2224	2.42	In-house DB
Levulinic acid	115.0400	2.43	In-house DB
Glutathione reduced	306.0760	5.62	CD
L-Glutathione oxidized	611.1434	1.17	CD
Lipoxin A4	351.2171	2.43	In-house DB
Lipoxin B4	351.2174	10.39	In-house DB
Phenylalanine	164.0716	4.93	CD
Pyroglutamic acid	128.0352	1.35	In-house DB
Serine	104.0352	5.96	CD
Lysine	145.0981	0.75	In-house DB
Malic acid	133.0141	1.04	In-house DB
Maleic acid	115.0035	1.20	In-house DB
Malonic acid	103.0036	1.11	In-house DB
Maltose	341.1083	5.74	CD
Mannitol	181.0715	5.36	CD
Mesaconic acid	129.0192	0.89	CD
Methionine	148.0437	1.21	In-house DB

Monobutyl phthalate	221.0817	10.73	CD
N-Acetylalanine	130.0508	1.65	CD
N-Acetyl-alpha-D-glucosamine 1-phosphate	300.0485	0.84	In-house DB
N-Acetyl-D-Glucosamine	220.0823	0.95	In-house DB
N-Acetyl-L-glutamic acid	188.0562	1.22	In-house DB
N-Acetyl-L-glutamine	187.0722	4.61	CD
N-Acetylneuraminic acid	308.0982	0.91	CD
N-Acetyl-α-D-glucosamine 1-phosphate	300.0484	5.85	CD
Nicotinic acid	122.0246	1.16	In-house DB
N-α-Acetyl-L-asparagine	173.0565	5.79	
O-Phosphoethanolamine	140.0117	0.82	In-house DB
O-Phosphorylethanolamine	140.0117	6.22	CD
Ornithine	131.0824	0.78	In-house DB
Orotic acid	155.0097	4.85	CD
Oxalic acid	88.9879	0.89	In-house DB
Pantothenic acid	218.1032	3.97	CD
Phosphoenolpyruvate	166.9749	0.90	In-house DB
Phosphoenolpyruvic acid	166.9750	6.03	CD
Prostaglandin G2	367.2113	2.63	In-house DB
Pyruvate	87.0086	1.07	In-house DB
Quinolinic Acid	166.0147	1.19	In-house DB
Ribose	149.0454	4.81	CD
Salicylic acid	137.0243	9.35	In-house DB
Succinate	117.0193	1.61	In-house DB
Sucrose	341.1083	0.89	In-house DB
Taurine	124.0072	0.85	In-house DB
Taurochenodeoxycholic acid	498.2890	9.71	CD
Thiamine	263.0967	0.78	In-house DB
Threonine	118.0508	0.86	In-house DB
trans-4-Hydroxy-L-proline	130.0508	0.89	In-house DB
trans-Ferulic acid	193.0506	2.43	In-house DB
Tryptophan	203.0825	5.12	CD
Tyrosine	180.0665	1.20	In-house DB
UMP	323.0279	1.08	In-house DB
Uracil	111.0199	1.15	CD
Uric acid	167.0209	1.12	CD
Uridine	243.0618	4.57	CD
Valine	116.0716	1.05	In-house DB
Xanthine	151.0260	1.32	CD

Xanthosine	283.0679	4.72	CD
α-D-Mannose 1-phosphate	259.0219	6.02	CD
β-D-Glucopyranuronic acid	193.0351	6.05	CD
δ-Ribono-1,4-lactone	147.0297	5.91	CD
Y-Aminobutyric acid (GABA)	102.0559	5.91	CD

[a] In-house database

[b] Compound Discoverer database

Table S3. Details of metabolites identified by MS/MS matching in 2DLC-MS (+) mode

Name	*m/z*	Retention time (min)	MS/MS database
1-Methyl-L-histidine	170.0920	0.75	In-house DB[a]
2-Aminobutyric acid	104.0704	5.39	CD[b]
3-Aminobutanoic acid	104.0704	0.87	In-house DB
3-Hydroxypyridine	96.0442	0.93	In-house DB
4-Acetamidobutanoic acid	146.0810	5.38	CD
4-Guanidinobutyric acid	146.0922	1.03	CD
4-Indolecarbaldehyde	146.0599	5.08	CD
4-Methyl-5-thiazoleethanol	144.0476	6.18	CD
5-Aminovaleric acid	118.0861	0.88	In-house DB
5-Methylcytosine	126.0660	1.19	CD
Alanine	90.0548	0.84	In-house DB
alpha-Aminoadipic acid	162.0757	0.93	In-house DB
Arginine	175.1187	7.18	CD
Asparagine	133.0606	5.84	CD
Aspartic acid	134.0446	6.23	CD
Betaine	118.0861	5.06	CD
Carnitine	162.1123	4.31	CD
Choline	104.1068	0.83	CD
cis-4-Hydroxy-D-proline	132.0655	5.57	CD
Citrulline	176.1027	0.85	In-house DB
Creatine	132.0766	5.85	CD
Cystathionine	223.0738	0.81	In-house DB
Cystine	241.0303	6.29	CD
Cytosine	112.0504	6.22	CD
D-Serine	106.0497	6.02	CD
Ethanolamine	62.0601	0.80	In-house DB
Glucose 1-phosphate	261.0361	6.28	CD
Glutamic acid	148.0604	5.88	CD
Glutamine	147.0762	0.86	In-house DB
Glycine	76.0392	0.81	In-house DB
Histidine	156.0765	0.79	In-house DB
Homoserine	120.0655	5.74	CD
Hypotaurine	110.0269	5.72	CD
Hypoxanthine	137.0456	4.62	CD
Isoleucine	132.1017	1.75	In-house DB
Leucine	132.1019	1.87	In-house DB
Leucylproline	229.1539	4.81	CD

Lysine	147.1126	7.04	CD
Methionine	150.0582	1.20	In-house DB
Methylimidazoleacetic acid	141.0658	5.74	CD
N3,N4-Dimethyl-L-arginine	203.1497	6.15	CD
N6,N6,N6-Trimethyl-L-lysine	189.1595	6.31	CD
N6-Acetyl-L-lysine	189.1231	1.02	CD
N-Acetyl-D-galactosamine	222.0968	5.59	CD
N-Acetyl-D-Glucosamine	222.0965	0.92	In-house DB
N-Acetyl-L-glutamic acid	190.0708	1.44	In-house DB
N-Acetyl-α-D-glucosamine	222.0967	5.23	CD
Nicotinamide	123.0551	1.18	CD
Nicotinic acid	124.0391	1.17	In-house DB
O-Phosphoethanolamine	142.0261	0.82	In-house DB
Ornithine	133.0969	0.75	In-house DB
Phenylalanine	166.0860	3.00	In-house DB
Phosphoenolpyruvate	168.9894	0.92	In-house DB
Pipecolinic acid	130.0860	0.75	CD
Proline	116.0704	5.31	CD
Pyridoxal	168.0651	0.92	CD
Pyroglutamic acid	130.0497	5.74	CD
Serine	106.0497	0.83	In-house DB
Stachydrine	144.1017	0.91	CD
Taurine	126.0219	5.61	CD
Threonine	120.0653	0.85	In-house DB
Tryptophan	205.0967	5.08	CD
Tyrosine	182.0810	1.44	In-house DB
Uracil	113.0344	4.53	CD
Urea	61.0397	0.92	In-house DB
Uric acid	169.0353	5.28	CD
Urocanic acid	139.0500	1.03	CD
Valine	118.0861	4.33	In-house DB
Y-Aminobutyric acid (GABA)	104.0704	6.11	CD

[a] In-house database

[b] Compound Discoverer database

Table S4. Metabolites in rat liver detected with significant changes in their abundance levels between G1 and G2 by different platforms

Metabolite ID	Metabolite name	*p*-value	Fold-change
GC×GC-MS			
1	1,2-Benzisothiazol-3-amine	4.3E-04	0.8
2	1-Butanol	2.2E-02	0.9
3	2-Pyrrolidone-5-carboxylic acid	7.4E-04	0.5
4	3-Aminobutyric acid	1.9E-02	0.6
5	3-Hydroxy-3-phenylpropionic acid	1.5E-02	1.4
6	3-Hydroxybutyric acid	2.5E-02	0.5
7	5-chloroindoline-2,3-dione	2.6E-02	0.7
8	5-Hexen-1-ol	3.0E-02	0.7
9	Acetimidamide	3.5E-02	0.6
10	Alanylglycine	9.3E-03	1.9
11	Benzoic Acid	2.7E-02	1.3
12	cis-11,14-Eicosadienoic acid	1.2E-02	1.8
13	DL-Glyceraldehyde	1.0E-02	0.1
14	Doconexent	5.5E-04	1.4
15	Eicosapentaenoic Acid	2.1E-02	2.2
16	Ethanolamine	3.8E-03	0.5
17	Heptadecanoic acid	3.4E-04	1.8
18	Hypoxanthine	8.6E-04	0.7
19	Isobutyl alcohol	9.1E-05	0.7
20	L-Aspartic acid	5.4E-04	0.5
21	L-Glutamine	4.5E-02	0.6
22	L -Leucine	4.5E-02	0.7
23	L -Lysine	8.5E-03	2.0
24	L -Malic acid	5.0E-02	1.6
25	L -Ornithine	4.1E-02	0.6
26	L -Phenylalanine	2.8E-02	0.6
27	L -Pyroglutamic acid	2.9E-02	0.7
28	L -Threonine	7.3E-05	0.4
29	Maleic acid	4.1E-03	1.5
30	Methylmalonic acid	5.7E-03	0.7
31	Niacinamide	3.2E-03	0.7
32	Nonanoic acid	1.2E-04	2.0
33	Octadeca-9,12-diynoic acid	9.8E-03	2.6
34	O-phosphoethanolamine	1.6E-03	0.5

35	Oxalic acid	7.9E-03	2.2
36	Propylene glycol	1.6E-02	1.7
37	pyruvate	1.3E-02	0.5
38	Stearic acid	9.7E-04	1.3
39	Taurine	4.6E-04	5.6
40	Uracil	4.4E-02	1.2
41	α-Aminobutyric acid	5.6E-04	0.4
2DLC-MS (-)			
1	2-Methylglutaric acid	8.7E-03	2.3
2	4-Methyl-2-oxovaleric acid	3.1E-02	0.7
3	4-Oxoproline	3.9E-05	0.6
4	5-CMP	4.0E-02	1.4
5	Hypotaurine	1.4E-03	2.2
6	L -Glutathione oxidized	1.2E-04	2.0
7	L -Malic acid	4.9E-02	1.3
8	L -Ornithine	4.1E-02	0.7
9	N-acetylalanine	1.1E-02	1.5
10	Pyruvic acid	4.7E-02	0.5
11	Taurine	1.7E-03	1.8
12	Taurochenodeoxycholic acid	7.8E-05	8.4
13	α-Ketoglutaric acid	9.8E-03	0.4
2DLC-MS (+)			
1	4-Acetamidobutanoic acid	1.1E-02	1.4
2	4-Guanidinobutyric acid	6.5E-05	2.9
3	Carnitine	1.0E-02	0.8
4	Cytosine	4.7E-02	10.9
5	Hypotaurine	1.6E-03	2.3
6	L -Ornithine	3.2E-02	0.9
7	L -Phenylalanine	2.4E-02	0.7
8	N-acetyl-D-Glucosamine	4.5E-02	1.5
9	Proline	9.2E-03	0.9
10	Serine	1.8E-02	0.9
11	Taurine	3.2E-02	1.7

Table S5. Detection of metabolites from the "biosynthesis of unsaturated fatty acid pathway" by different platforms.

Compound Name	GC×GC-MS	2DLC-MS(-)	2DLC-MS(+)
α-linolenic acid, ALA	x[a]	x	x
Stearidonic acid	x	x	y[b]
Arachidonic acid	y	y	x
Eicosapentaenoic acid, EPA	y	y	y
Docosapentaenoic acid (DPA)	x	y	y
Docosahexaenoic acid, DHA	y	x	x
Linoleic acid, LA	y	x	x
Gamma-linolenic acid, GLA	y	x	x
Dihomogamma-linolenic acid, DGLA	y	x	y
PG2 (prostaglandin G2)	x	y	x
TXA2 (thromboxane A2)	x	x	x
leukotriene B4	x	y	x
LXA4	x	y	x
LXB4	x	y	x

[a]x stands for a compound was not detected in the corresponding platform.

[b]y stands for a compound was detected in the corresponding platform.