SUPPLEMENTARY MATERIALS

NMR-based Metabolomics with Enhanced Sensitivity

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Table S1: Embryo medium composition

Sr No	Metabolite
1	Sodium chloride
2	Potassium chloride
3	Magnesium sulfate
4	Potassium phosphate
5	Glucose, D-(+)
6	Sodium pyruvate
7	L-Asparagine
8	L-Aspartic acid
9	Glycine
10	L-Proline
11	L-Serine
12	L-Glutamic acid
13	L-Arginine
14	L-Cystine
15	L-Histidine
16	L-Isoleucine
17	L-Lysine
18	L-Methionine
19	L-Phenylalanine
20	L-Threonine
21	L-Tryptophan
22	L-Tyrosine
23	L-Valine
24	L- leucine

Table S2: Acquisition parameters

Sample	Condition	Experiment	Spectral Width (ppm)		Offset (ppm)		Acquisition time (t_{max}) (ms)		No.of Complex points		Scans
			F1 (^{13}C)	F2 (^{1}H)	F1 (^{13}C)	F2 (^{1}H)	F1 (^{13}C)	F2 (^{1}H)	F1 (^{13}C)	F2 (^{1}H)	
Urine	CTO+5mm	HSQC	75	12	40	4.7	8.5	53.2	512	1024	8
	SNTO+shaped tube	HSQC	75	12	40	4.7	8.5	53.2	512	1024	8
Media	CTO+5mm	HSQC	75	12	40	4.7	8.5	53.2	512	1024	8
	SNTO+shaped tube	HSQC	75	12	40	4.7	8.5	53.2	512	1024	8
Bovine Serum	CTO+5mm	HSQC	75	12	40	4.7	8.5	53.2	512	1024	8
	SNTO+shaped tube	HSQC	160	12	75	4.7	4.7	90.1	360	2048	32
	SNTO+shaped tube	HSQC	160	12	75	4.7	4.7	90.1	360	2048	32

Table S3a: The Signal-to-Noise ratio for different peaks in case bovine serum for a one-dimensional experiment with excitation sculpting water suppression. The data was recorded in a conventional 5 mm tube at CTO position and in shaped tube at SNTO position on a 950 MHz spectrometer.

Chemical Shift (ppm)	**Assignment**	**SNR (CTO +5mm)**	**SNR (CTO +Shape Tube)**	**SNR (SNTO +Shaped Tube)**	**SNR Volume adjusted(SNTO +Shaped Tube)**	**Ratio of SNR (SNTO +Shaped Tube)/ (CTO + 5mm)**	**Ratio of SNR Volume adjusted (SNTO +Shaped Tube)/ SNR (CTO + 5mm)**
5.25	H^{α}-Glucose	967.4	1056.1	1120.6	3361.8	1.16	3.44
5.4	Allantoin	499.4	547.5	574.3	1723.2	1.15	3.44
8.46	Formate	490.6	541.4	574.6	1723.8	1.17	3.51
1.04	Valine	1383.9	1407.2	1421.1	4263.3	1.03	3.08
2.41	Succinate	6102.1	6106.7	6127.6	18382.8	1.01	3.01
	Average					1.10	3.30

Table S3b: The signal to noise ratio of five distinct peaks of media spectra recorded in a conventional 5 mm tube at CTO position and in shaped tube at SNTO position on an 800 MHz spectrometer. The noise region was kept the same in all the analyses (13.5- 12.8 ppm).

Chemical Shift (ppm)	Assignment	SNR (CTO +5mm)	SNR (CTO + Shapetube)	SNR (SNTO +Shaped Tube)	SNR Volume adjusted (SNTO +Shaped Tube)	Ratio of SNR (SNTO +Shaped Tube)/ (CTO + 5mm)	The ratio of SNR (SNTO +Shaped Tube)/ SNR (CTO + 5mm)
7.785	Histidine	7.3	7.9	10	30	1.37	4.11
7.624	Aspargine	19.8	20.9	25.6	76.8	1.30	3.88
7.312	Phenylalanine	20.3	21.7	24.3	72.9	1.20	3.59
7.225	Phenylalanine	27.5	28.6	31.7	95.1	1.15	3.46
6.917	Aspargine	24.4	26.7	31.2	93.6	1.28	3.84
Average						1.26	3.78

Table S3c: The signal to noise ratio of five distinct peaks of urine spectra recorded in a conventional 5 mm tube at CTO position and in shaped tube at SNTO position on an 800 MHz spectrometer. The noise region was kept the same in all the analyses of S/N (10.5- 9.8 ppm).

Chemical Shift (ppm)	Assignment	SNR (CTO +5mm)	SNR (SNTO +Shaped Tube)	SNR Volume adjusted (SNTO +Shaped Tube)	Ratio of SNR (SNTO +Shaped Tube)/ (CTO + 5mm)	The ratio of SNR (SNTO +Shaped Tube)/ SNR (CTO + 5mm)
7.66	Pyridoxine	67.1	70.4	211.2	1.05	3.12
7.73	Indole-3-lactate	37.4	42.1	126.3	1.13	3.38
7.45	Hippurate	36.7	40.0	120	1.09	3.27
7.53	Hippurate	19.1	20.9	62.7	1.09	3.28
1.36	Isoleucine	107.4	116.8	350.4	1.09	3.26
Average					1.09	3.27

Figure S1: Figure S2a represents the 2D [^{1}H, ^{13}C] HSQC (Heteronuclear Single Quantum Correlation) spectra of media at SNTO with a shaped tube. Figure S2b represents the 2D [^{1}H, ^{13}C] HSQC (Heteronuclear Single Quantum Correlation) spectra of media at CTO with 5mm tube.

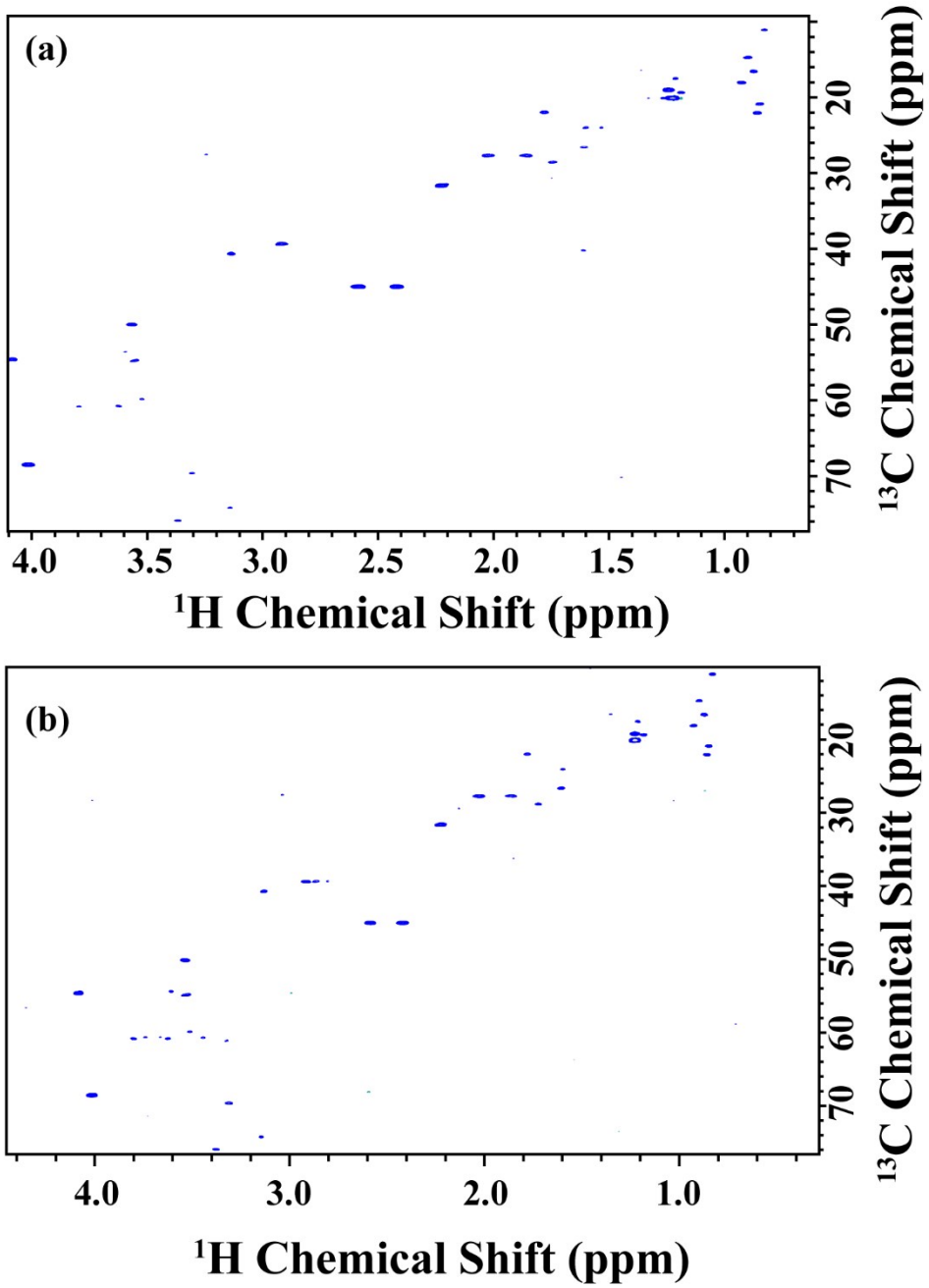

Figure S2: Figure S3a represents the 2D [^{1}H, ^{13}C] HSQC (Heteronuclear Single Quantum Correlation) spectra of Urine at SNTO with a shaped tube. Figure S3b represents the 2D [^{1}H, ^{13}C] HSQC (Heteronuclear Single Quantum Correlation) spectra of media at CTO with 5mm tube.

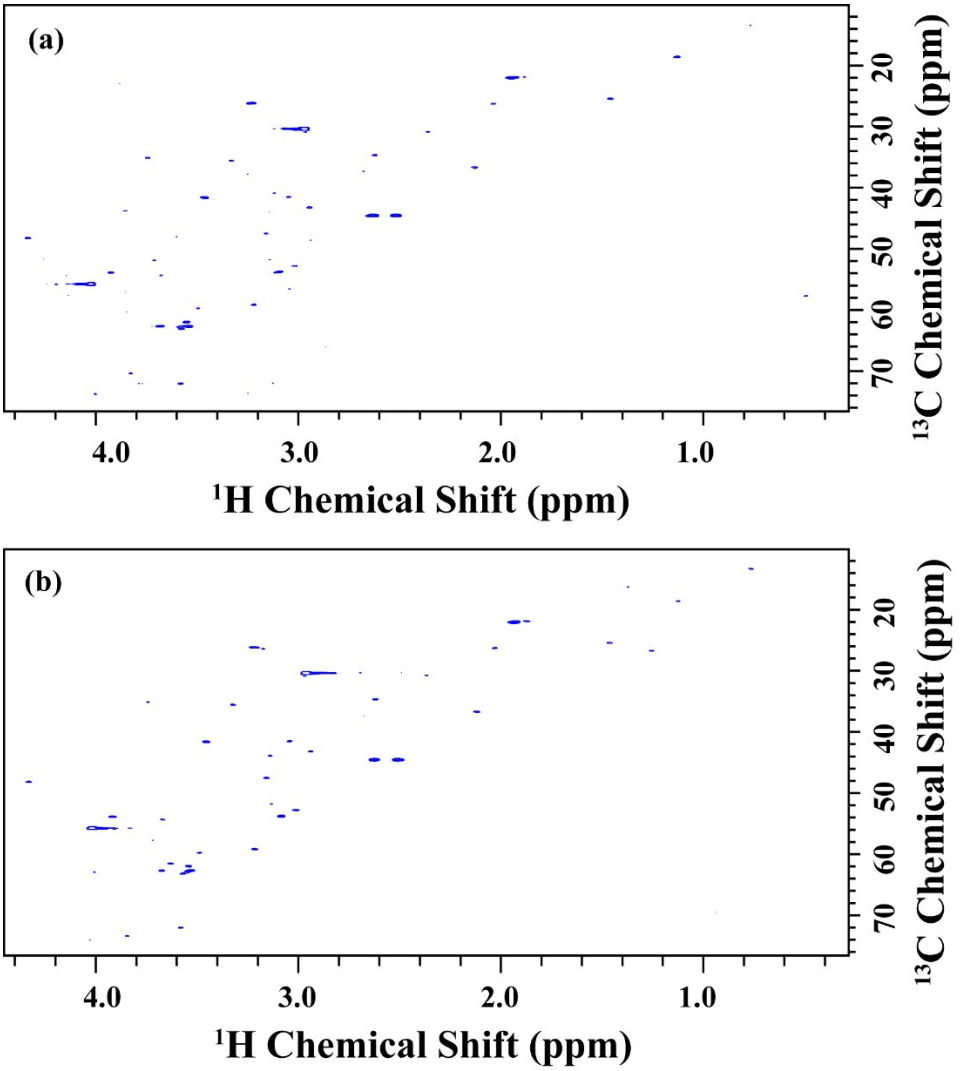

Table S4: Comparison of Bovine Serum 2D [^{1}H, ^{13}C] HSQC at the conditions (I) CTO+5mm tube (II) SNTO + shaped tube. The lower panel shows the average S/N and average sensitivity gain considering the volume effect.

Sr No	Assignment	Carbon Chemical Shift(ppm)	Proton Chemical Shift(ppm)	SNR (5mm Tube+CTO)	SNR (Shaped Tube+SNTO)	SNR volume adjusted (Shaped Tube+ SNTO)	SNR(Shaped Tube + SNTO)/SNR(5mm+ CTO)	SNR volume adjusted (Shaped Tube + SNTO)/SNR (5mm+ CTO)
1	Fructose	82.174	4.10	131	179	537	1.37	4.10
2	D-Glucuronic acid	80.708	3.82	431	549	1647	1.27	3.82
3	Mannose	76.221	4.24	104	147	441	1.41	4.24
4	Glycoholic acid	75.691	3.07	86	88	264	1.02	3.07
5	Rhamnose	74.633	3.69	548	674	2022	1.23	3.69
6	Fucose	74.36	3.97	746	986	2958	1.32	3.97
7	Glycoholic acid	74.458	3.81	267	339	1017	1.27	3.81
8	Metoprolol	75.961	3.63	190	230	690	1.21	3.63
9	D-Pantethine	71.461	3.93	595	780	2340	1.31	3.93
10	Pantothenic acid	71.307	4.76	63	100	300	1.59	4.76
11	Pantothenic acid	71.258	3.86	331	426	1278	1.29	3.86
12	Mannose	72.658	3.52	431	505	1515	1.17	3.52
13	Fucose	72.183	3.98	278	369	1107	1.33	3.98
14	Rhamnose	72.857	3.38	456	513	1539	1.13	3.38
15	Fructose	73.105	3.90	353	459	1377	1.30	3.90
16	Fructose	72.208	3.45	185	213	639	1.15	3.45
17	Xylose	71.225	3.67	275	336	1008	1.22	3.67
18	Glycoholic acid	71.54	4.00	406	541	1623	1.33	4.00
19	Fructose	71.909	3.60	449	538	1614	1.20	3.60
20	Fructose	72.341	3.38	354	399	1197	1.13	3.38
21	Fucose	69.358	3.84	1976	2529	7587	1.28	3.84
22	Metoprolol	71.909	3.60	449	538	1614	1.20	3.60
23	Cholic acid	69.771	3.41	341	388	1164	1.14	3.41
24	cholic acid	69.812	3.18	215	228	684	1.06	3.18
25	Acetylcarnitine	69.81	3.78	1002	1264	3792	1.26	3.78
26	Mannose	68.587	2.65	94	83	249	0.88	2.65
27	Xylose	67.728	2.43	188	152	456	0.81	2.43
28	Acetylcholine	67.527	3.48	124	144	432	1.16	3.48
29	Glyceric acid	67.617	3.23	128	138	414	1.08	3.23
30	Uridine diphosphate glucose	67.201	4.42	19	28	84	1.47	4.42
31	Uridine diphosphate glucose	67.224	5.08	13	22	66	1.69	5.08
32	Metoprolol	67.371	4.40	15	22	66	1.47	4.40

33	Thiamine pyrophosphate	66.203	3.89	27	35	105	1.30	3.89
34	D-Arabitol	64.699	3.60	9	37	111	4.11	12.33
35	Riboflavin	66.234	3.38	64	80	240	1.25	3.75
36	Riboflavin	66.915	3.84	14	23	69	1.64	4.93
37	Xylose	67.762	3.60	1066	1340	4020	1.26	3.77
38	Acetylcarnitine	69.179	3.41	13	38	114	2.92	8.77
39	Acetylcarnitine	69.669	3.18	17	26	78	1.53	4.59
40	Cholic acid	69.059	3.78	94	14	42	0.15	0.45
41	Mannose	69.73	2.65	853	1114	3342	1.31	3.92
42	Mannose	69.714	2.43	146	280	840	1.92	5.75
43	Mannose	69.707	3.48	73	142	426	1.95	5.84
44	Betaine	69.171	3.23	94	124	372	1.32	3.96
45	Glycerophosphocholine	68.214	4.42	252	311	933	1.23	3.70
46	Xylose	68.214	5.08	252	311	933	1.23	3.70
47	Mannose	68.473	4.40	225	253	759	1.12	3.37
48	Glyceric acid	67.482	3.89	62	88	264	1.42	4.26
49	Mannose	65.998	12.33	39	47	141	1.21	3.62
50	D-Arabitol	65.882	3.75	12	19	57	1.58	4.75
51	Xylose	66.854	4.93	6	17	51	2.83	8.50
52	Choline	59.639	3.60	27	35	105	1.30	3.89
53	Creatine	59.682	3.38	22	30	90	1.36	4.09
54	Creatinine	59.661	3.84	33	35	105	1.06	3.18
55	Lisinopril	61.284	3.60	64	80	240	1.25	3.75
56	Seine	63.413	3.41	768	824	2472	1.07	3.22
57	Maltitol	62.196	3.18	8	43	129	5.38	16.13
58 *	Unassigned	60.377	3.78	79	93	279	1.18	3.53
59	Cyanocobalamin	61.588	2.65	46	52	156	1.13	3.39
60	Glucose-1-phosphate	62.821	2.43	369	500	1500	1.36	4.07
61	Glucose-1-phosphate	62.875	3.48	172	208	624	1.21	3.63
62	Ethanol	60.747	3.23	308	349	1047	1.13	3.40
63	Homoserine	61.297	4.42	104	116	348	1.12	3.35
64	Dimethylglycine	62.478	5.08	268	394	1182	1.47	4.41
65	N-Methyl D-Aspartic acid	62.758	4.40	154	194	582	1.26	3.78
66	Deoxyguanosine	62.57	3.89	152	195	585	1.28	3.85
67	Trehalose	63.989	12.33	806	1046	3138	1.30	3.89
68	Valine	63.46	3.75	748	1001	3003	1.34	4.02
69	Deoxyguanosine	62.614	4.93	868	527	1581	0.61	1.82
70	Isoleucine	62.614	1.82	868	527	1581	0.61	1.82
71	Serine	59.777	4.00	33	44	132	1.33	4.00

72	Pipecolic acid	61.608	4.29	35	50	150	1.43	4.29
73	Uridine diphosphate glucose	62.881	3.65	349	424	1272	1.22	3.65
74	Pseudophedrine	62.832	4.08	441	600	1800	1.36	4.08
75	Threonine	64.042	4.18	784	1092	3276	1.39	4.18
76	Cyanocobalamin	60.747	3.40	308	349	1047	1.13	3.40
77	2-Methylacetoacetic acid	51.859	4.04	210	283	849	1.35	4.04
78	Lisinopril	50.727	3.79	102	129	387	1.27	3.79
Average Sensitivity gain							**1.38**	**4.15**

Table S5: Comparison of Urine 2D [^{1}H, ^{13}C] HSQC at the conditions (I) CTO+5mm tube (II) SNTO + shaped tube. The lower panel shows the average S/N and average sensitivity gain considering the volume effect.

Sr N0	Assignment	Proton Chemical Shift(ppm)	Carbon Chemical Shift(ppm)	SNR (5mm Tube+CTO)	SNR (Shaped Tube+SNTO)	SNR adjested volume (Shaped Tube+SNTO)	SNR(Shaped Tube + SNTO)/SNR(5mm+ CTO)	SNR volume adjusted (Shaped Tube + SNTO)/SNR(5mm+ CTO)
1	Isoleucine	1.368	8.24	6	16.48	49.44	2.75	8.24
2	α-ketoisovaleric acid	1.121	2.09	28.85	20.14	60.42	0.70	2.09
3	Androsterone	1.937	3.24	40.85	44.16	132.48	1.08	3.24
4	Isoleucine	1.455	2.08	20	13.83	41.49	0.69	2.08
5	Proline	2.031	3.18	14.57	15.46	46.38	1.06	3.18
6	Cholesterol	1.209	4.19	7.42	10.37	31.11	1.40	4.19
7	Proline	2.342	3.16	13.14	13.83	41.49	1.05	3.16
8	1-methylhistamine	3.222	2.48	28.57	23.6	70.8	0.83	2.48
10	Homocarnosine	2.964	2.62	1120.29	979.69	2939.07	0.88	2.62
11	Carnosine	2.964	3.05	16	16.28	48.84	1.02	3.05
12	Asparagine	3.738	2.38	20	15.87	47.61	0.79	2.38
13	Methionine	3.321	2.74	17.14	15.67	47.01	0.91	2.74
14	Carnosine	2.617	3.10	25.42	26.25	78.75	1.03	3.10
15	Glycoholic acid	2.119	3.09	18	18.51	55.53	1.03	3.09
16	Lysine	3.456	2.25	62.28	46.8	140.4	0.75	2.25
17	6-Dimethylaminopurine	3.04	4.07	20.57	27.88	83.64	1.36	4.07
18	Agmatine	2.937	2.49	27.42	22.79	68.37	0.83	2.49
19	Succinate	2.624	2.87	77.14	73.87	221.61	0.96	2.87
20	Methyl succinic acid	2.512	2.75	77.71	71.22	213.66	0.92	2.75
21	Adenosylcobalamin	3.151	3.19	16.28	17.29	51.87	1.06	3.19
22	Proline	4.331	3.01	23.71	23.81	71.43	1.00	3.01
23	"3-Hydroxyisovalerate,"	3.011	3.45	14.85	17.09	51.27	1.15	3.45
24	"3-Hydroxyisovalerate,"	3.04	3.71	9.71	12	36	1.24	3.71
25	Carnatine	3.084	3.24	38.85	41.92	125.76	1.08	3.24

26	Alanine	3.92	3.07	22.28	22.79	68.37	1.02	3.07
27	Leucine	4.02	2.65	868.28	765.39	2296.17	0.88	2.65
28	Histidine	3.216	2.85	25.71	24.421	73.263	0.95	2.85
29	Adenosylcobalamin	3.679	2.40	30.57	24.42	73.26	0.80	2.40
30	Valine	3.565	2.43	16.57	13.43	40.29	0.81	2.38
31	Pipecolic acid	3.532	2.12	36.28	25.64	76.92	0.71	2.43
32	Isoleucine	3.544	2.77	35.71	32.96	98.88	0.92	2.12
34	Ethanolamine	3.885	2.95	9.71	9.56	28.68	0.99	2.77
35	Sucrose	3.433	5.76	6.57	12.61	37.83	1.92	2.95
36	Cholesterol	3.58	2.79	23.42	21.77	65.31	0.93	5.76
37	Melibiose	3.993	2.38	12.28	9.76	29.28	0.80	2.79
38	Diphenhydramine	3.492	1.69	15.14	8.54	25.62	0.56	2.38
40	Asparagine	3.931	3.20	8.57	9.15	27.45	1.07	1.69
Average Sensitivity gain							1.03	3.07

Table S6: Comparison of media 2D [^{1}H, ^{13}C] HSQC at the conditions (I) CTO+5mm tube (II) SNTO + shaped tube. The lower panel shows the average S/N and average sensitivity gain considering the volume effect.

Sr No	**Assignment**	**Carbon Chemical Shift(ppm)**	**Proton Chemical Shift(ppm)**	**SNR (5mm Tube+CTO)**	**SNR (Shaped Tube+SNTO)**	**SNR volume adjusted (Shaped Tube+ SNTO)**	**SNR(Shaped Tube + SNTO)/SNR(5mm + CTO)**	**SNR volume adjusted (Shaped Tube + SNTO)/SNR (5mm+ CTO)**
1	Isoleucine	17.588	0.993	30.0971	43.1169	129.351	1.43	4.30
2	Valine	19.445	0.968	38.2524	43.3766	130.13	1.13	3.40
3	Valine	20.95	1.024	37.4757	49.6104	148.831	1.32	3.97
4	Leucine	23.763	0.944	32.4272	40.7792	122.338	1.26	3.77
5	Leucine	24.932	0.956	43.4951	44.1558	132.467	1.02	3.05
6*	Unassigned	22.222	1.278	33.9806	38.1818	114.545	1.12	3.37
7	Threonine	22.08	1.325	168.155	188.052	564.156	1.12	3.36
8	Alanine	20.395	1.309	23.301	24.6753	74.0259	1.06	3.18
9*	Unassigned	22.958	1.325	759.029	864.416	2593.25	1.14	3.42
10	Lysine	24.862	1.877	30.2913	36.3636	109.091	1.20	3.60

11	Leucine	26.918	1.694	14.5631	18.961	56.883	1.30	3.91
12	Lysine	29.481	1.701	23.6893	17.4026	52.2078	0.74	2.20
13	Lysine	31.686	1.818	18.2524	23.1169	69.3507	1.27	3.80
14	Valine	30.571	1.959	34.9515	43.3766	130.13	1.24	3.72
15	Pyruvate	30.578	2.123	43.1068	45.974	137.922	1.07	3.20
16	Glycine	43.563	3.229	35.534	45.974	137.922	1.29	3.88
17	Alanine	52.943	3.631	68.3495	71.1688	213.506	1.04	3.12
18	Leucine	57.205	3.706	13.9806	13.5065	40.5195	0.97	2.90
19	Lysine	57.71	3.625	31.2621	28.0519	84.1557	0.90	2.69
20	Serine	63.643	3.896	17.4757	15.8442	47.5326	0.91	2.72
21	Threonine	71.413	4.112	159.223	168.052	504.156	1.06	3.17
22	Glucose	77.058	3.244	15.534	17.1429	51.4287	1.10	3.31
23	Glucose	78.758	3.479	13.7864	19.4805	58.4415	1.41	4.30
Average Sensitivity gain							1.13	3.40