Electronic supplementary information (ESI)

Merging metabolomics and lipidomics into one analytical run

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Table S1. List of metabolites, retention times, sum formulas, detected *m/z*, limits of detection for an injection volume of 5 µL, recoveries of metabolite concentrations in a 1 µM QC sample (giving the trueness bias) based on internal standardization with fully ^{13}C labeled yeast extract, linear calibration ranges and corresponding correlation coefficients for both positive and negative electrospray ionization.

Compound	RT [min]	Formula	$[M+H]^+$	$[M-H]^-$	LOD pos [µM]	LOD neg [µM]	ISTD pos	ISTD neg	Recovery pos [%]	Recovery neg [%]	Linear range pos	R^2 pos	Linear range neg	R^2 neg
1-Methylnicotinamide	9.93	C7H8N2O	137.0709	135.0564	0.02		External		100		0.01 - 10	1.0000		
2-Carbamoylamino-butanedioic acid	4.86	C5H8N2O5	177.0506	175.0360	0.2	0.06	External	External	118	107	0.1 - 5	0.9976	0.01 - 10	0.9979
2-Deoxycytidine	2.17	C9H13N3O4	228.0979	226.0833	0.03	0.08	U13C Adenosine	U13C AMP	118	119	0.01 - 10	0.9955	0.05 - 10	0.9949
2-Deoxyuridine	1.55	C9H12N2O5	229.0819	227.0673		0.02		U13C Adenine		128			0.05 - 10	0.9983
3AMP	2.90	C10H14N5O7P	348.0704	346.0558	0.10	0.07	U13C AMP	U13C AMP	109	128	0.1 - 10	0.9990	0.05 - 25	0.9981
3-Methyl-2-oxovaleric acid	0.98	C6H10O3	131.0703	129.0557		0.05		U13C 3-Methyl-2-oxovaleric acid		100			0.01 - 10	0.9981
3-Methylcytidine	4.08	C10H15N3O5	258.1085	256.0939	0.06	0.1	External	U13C Pseudouridine	97	78	0.01 - 1	0.9998	0.5 - 10	0.9951
3-Phosphoglycerate	5.34	C3H7O7P	187.0002	184.9857		0.02		U13C 3-Phosphoglycerate		97			0.1 - 10	0.9998
4-Hydroxy-proline	3.65	C5H9NO3	132.0655	130.0510	0.5	0.08	External	U13C AMP	137	109	0.01 - 10	0.9968	0.5 - 25	0.9979
5-Deoxy-5-Methylthioadenosine	1.24	C11H15N5O3S	298.0968	296.0823	0.02	0.05	U13C 5-Deoxy-5-Methylthioadenosine	U13C 5-Deoxy-5-Methylthioadenosine	103	96	0.01 - 25	0.9994	0.5 - 10	0.9914
5-Methyluridine	1.59	C10H14N2O6	259.0925	257.0779	0.2	0.1	External	External	114	120	0.5 - 5	1.0000	0.05 - 5	0.9970
6-Phosphogluconate	5.73	C6H13O10P	277.0319	275.0174	0.05	0.1	External	U13C 6-Phosphogluconate	92	102	0.5 - 25	0.9957	0.1 - 25	0.9990
Adenine	1.86	C5H5N5	136.0618	134.0472	0.02	0.05	U13C Adenine	U13C Adenine	98	95	0.01 - 10	0.9995	0.1 - 10	0.9963
Adenosine	1.70	C10H13N5O4	268.1040	266.0895	0.03	0.09	U13C Adenosine	U13C Adenine	99	109	0.01 - 25	0.9998	0.1 - 10	0.9968
ADP	4.43	C10H15N5O10P2	428.0367	426.0221	0.04	0.2	U13C ADP	U13C ADP	106	111	0.05 - 25	1.0000	0.5 - 25	0.9965
Alanine	3.76	C3H7NO2	90.0550	88.0404	0.1	0.1	U13C Alanine	U13C Alanine	120	144	0.05 - 25	0.9992	0.5 - 25	0.9970
alpha-Aminoadipic acid	3.73	C6H11NO4	162.0761	160.0615	0.04	0.01	U13C alpha-Aminoadipic acid	U13C alpha-Aminoadipic acid	105	102	0.01 - 10	0.9992	0.1 - 25	0.9992
alpha-Ketoglutarate	4.14	C5H6O5	147.0288	145.0143		0.02		U13C alpha-Ketoglutarate		114			0.01 - 25	0.9990
AMP	3.24	C10H14N5O7P	348.0704	346.0558	0.004	0.06	U13C AMP	U13C AMP	99	106	0.05 - 25	0.9994	0.05 - 25	0.9997
Arginine	9.51	C6H14N4O2	175.1190	173.1044	0.02	0.02	U13C Arginine	U13C Arginine	109	109	0.05 - 25	0.9996	0.05 - 25	0.9992
Argininosuccinic acid	4.76	C10H18N4O6	291.1299	289.1154	0.04	0.04	U13C Argininosuccinic acid	U13C Argininosuccinic acid	102	104	0.01 - 25	0.9999	0.05 - 25	0.9998
Asparagine	3.97	C4H8N2O3	133.0608	131.0462	0.05	0.06	U13C Asparagine	U13C Asparagine	97	95	0.5 - 25	0.9994	0.1 - 25	0.9975
Aspartate	3.77	C4H7NO4	134.0448	132.0302	0.1	0.04	U13C Aspartate	U13C Aspartate	93	107	0.05 - 10	0.9988	0.01 - 25	0.9995
ATP	5.20	C10H16N5O13P3	508.0030	505.9885	0.06	0.08	U13C ATP	U13C ATP	111	103	0.05 - 25	0.9996	0.5 - 25	0.9992
Betaine	2.34	C5H11NO2	118.0863	116.0717	0.2		U13C Adenosine		88		0.05 - 5	0.9985	5 - 25	0.9978

Compound	RT [min]	Formula	$[M+H]^+$	$[M-H]^-$	LOD pos [µM]	LOD neg [µM]	ISTD pos	ISTD neg	Recovery pos [%]	Recovery neg [%]	Linear range pos	R^2 pos	Linear range neg	R^2 neg
Biotin	1.39	C10H16N2O3S	245.0954	243.0809	0.5	0.09	External	External	130	111	0.01 - 10	0.9934	0.01 - 10	0.9997
cAMP	1.62	C10H12N5O6P	330.0598	328.0452	0.05	0.05	U13C AMP	U13C AMP	102	109	0.01 - 10	0.9971	0.05 - 25	0.9996
Carnitine	3.07	C7H15NO3	162.1125	160.0979	0.2		U13C Adenosine	External	89		0.01 - 5	0.9985		
cGMP	2.91	C10H12N5O7P	346.0547	344.0402	0.09	0.05	U13C AMP	U13C GMP	105	135	0.05 - 10	0.9983	0.05 - 10	0.9961
Choline	7.95	C5H13NO	104.1070	102.0924	0.02		U13C Choline		106		0.01 - 10	0.9994		
cis-Aconitate	5.40	C6H6O6	175.0237	173.0092		0.01		U13C cis-Aconitate		108			0.01 - 25	0.9992
Citrate	5.77	C6H8O7	193.0343	191.0197		0.05		U13C Citrate		105			0.5 - 25	0.9981
Citrulline	4.30	C6H13N3O3	176.1030	174.0884	0.01	0.08	U13C Citrulline	U13C Citrulline	108	106	0.01 - 25	0.9998	0.5 - 25	0.9978
CMP	4.37	C9H14N3O8P	324.0591	322.0446	0.08	0.09	U13C CMP	U13C AMP	92	99	0.01 - 10	0.9981	0.5 - 25	0.9979
CTP	6.13	C9H16N3O14P3	483.9918	481.9772	0.1	0.1	U13C CTP	U13C CTP	104	100	0.1 - 10	0.9950	0.1 - 10	0.9996
Cystathionine	4.82	C7H14N2O4S	223.0747	221.0602	0.04	0.05	U13C Cystathionine	U13C Cystathionine	101	101	0.05 - 25	0.9998	0.05 - 25	0.9990
Cysteic acid	3.98	C3H7NO5S	170.0118	167.9972		0.04	External	U13C alpha-Ketoglutarate		200	5 - 25	0.9596	0.01 - 25	0.9974
Cysteine	n.d.	C3H7NO2S	122.0270	120.0125										
Cystine	4.35	C6H12N2O4S2	241.0311	239.0166	0.1		External		109		0.5 - 25	0.9913		
Cytidine	2.68	C9H13N3O5	244.0928	242.0782	0.1	0.06	U13C Adenosine	U13C AMP	86	117	0.01 - 10	0.9895	0.01 - 10	0.9964
Cytosine	2.43	C4H5N3O	112.0505	110.0360	0.3		U13C Adenine		125		0.05 - 10	0.9997		
dAMP	2.79	C10H14N5O6P	332.0755	330.0609	0.04	0.05	U13C AMP	U13C AMP	116	107	0.01 - 10	0.9983	0.01 - 25	0.9994
dATP	4.76	C10H16N5O12P3	492.0081	489.9936	0.16	0.05	U13C AMP	U13C AMP	80	82	0.05 - 5	0.9985	0.05 - 25	0.9976
dCMP	3.77	C9H14N3O7P	308.0642	306.0497	0.05	0.04	U13C AMP	U13C AMP	85	93	0.05 - 10	0.9947	0.05 - 25	0.9995
dCTP	5.67	C9H16N3O13P3	467.9969	465.9823	0.2	0.05	U13C AMP	U13C AMP	74	76	0.05 - 10	0.9920	0.05 - 25	0.9972
dGTP	6.04	C10H16N5O13P3	508.0030	505.9885	0.1	0.09	U13C AMP	U13C AMP	84	91	0.5 - 10	0.9951	0.5 - 25	0.9988
Dihydroxyacetone-phosphate	4.08	C3H7O6P	171.0053	168.9908		0.07		U13C Dihydroxyacetone-phosphate		102			0.5 - 10	0.9994
Dihydroxyisovalerate	1.15	C5H10O4	135.0652	133.0506		0.01		U13C Dihydroxyisovalerate		110			0.01 - 25	0.9997
Erythrol	2.58	C4H10O4	123.0652	121.0506		0.09		External		99			0.1 - 10	0.9967
Erythrose-4-phosphate	4.70	C4H9O7P	201.0159	199.0013		> 1 µM		U13C Hexose-P					5 - 25	0.9960
Flavinadenin dinucleotide	2.36	C27H33P2N9O15	786.1644	784.1499	0.1	0.04	U13C NAD+	U13C NAD+	124	119	0.05 - 10	0.9955	0.05 - 25	0.9997
Hexose	3.25	C6H12O6	181.0707	179.0561		0.40		U13C Hexose		115			0.4 - 100	0.9939
Fructose-1,6-bisphosphate	6.26	C6H14O12P2	341.0033	338.9888		0.1	U13C Fructose-1,6-bisphosphate	U13C Fructose-1,6-bisphosphate		115	5 - 25	0.9990	0.05 - 25	0.9990
F6P+G1P	4.49	C6H13O9P	261.0370	259.0224	0.23	0.18	U13C F6P+G1P	U13C Hexose-P	109	114	0.2 - 20	0.9977	0.1 - 50	0.9991
Fumarate	4.53	C4H4O4	117.0182	115.0037		0.03		U13C Fumarate		110			0.05 - 25	0.9996
GDP	5.84	C10H15N5O11P2	444.0316	442.0171	0.06	0.03	U13C GDP	U13C GDP	108	107	0.05 - 25	0.9999	0.05 - 25	0.9994

Compound	RT [min]	Formula	$[M+H]^+$	$[M-H]^-$	LOD pos [µM]	LOD neg [µM]	ISTD pos	ISTD neg	Recovery pos [%]	Recovery neg [%]	Linear range pos	R^2 pos	Linear range neg	R^2 neg
Gluconate	3.20	C6H12O7	197.0656	195.0510		0.02		U13C Gluconate		111			0.01 - 25	0.9993
Glucose-6-phosphate	4.99	C6H13O9P	261.0370	259.0224	0.1	0.02	U13C Glucose-6-phosphate	U13C Hexose-P	96	107	0.1 - 10	0.9994	0.1 - 25	0.9997
Glutamate	3.58	C5H9NO4	148.0604	146.0459	0.05	0.06	U13C Glutamate	U13C Glutamate	104	104	0.5 - 25	0.9998	0.1 - 25	0.9995
Glutamine	3.90	C5H10N2O3	147.0764	145.0619	0.08	0.07	U13C Glutamine	U13C Glutamine	105	102	0.5 - 25	0.9998	0.1 - 25	1.0000
Glutamyl-cysteine	3.32	C8H14N2O5S	251.0696	249.0551	0.2	0.07	U13C Glutamine	U13C Glutamate	114	124	0.5 - 10	0.9978	0.5 - 10	0.9998
Glutathione, oxidized	5.31	C20H32N6O12S2	613.1592	611.1447	0.06	0.07	U13C Glutathione, oxidized	U13C Glutathione, oxidized	108	99	0.05 - 25	0.9995	0.05 - 25	0.9985
Glutathione, reduced	3.45	C10H17N3O6S	308.0911	306.0765	0.07	0.1	U13C Glutathione, reduced	U13C Glutathione, reduced	106	104	0.05 - 25	0.9992	0.1 - 25	0.9989
Glycine	4.21	C2H5NO2	76.0393	74.0248		> 1 µM	U13C Glycine	U13C Glycine			5 - 25	0.9829	5 - 25	0.9998
GMP	4.83	C10H14N5O8P	364.0653	362.0507	0.02	0.02	U13C GMP	U13C GMP	103	105	0.01 - 10	0.9997	0.05 - 25	0.9993
GTP	6.52	C10H16N5O14P3	523.9979	521.9834	0.08	0.07	U13C GTP	U13C GTP	110	102	0.1 - 25	0.9991	0.5 - 25	0.9984
Guanidineacetic acid	4.22	C3H7N3O2	118.0611	116.0466	0.05	0.1	External	U13C Succinate	101	104	0.01 - 1	0.9996	0.5 - 25	0.9996
Guanine	2.80	C5H5N5O	152.0567	150.0421	0.1	0.06	U13C Adenosine	U13C Adenine	97	109	0.01 - 25	0.9973	0.05 - 10	0.9916
Guanosine	3.10	C10H13N5O5	284.0990	282.0844	> 0.5 µM	0.05	U13C Adenosine	U13C Adenine	146	104	0.01 - 10	0.9962	0.05 - 25	0.9946
Histidine	Insuffi-cient peak shape	C6H9N3O2	156.0768	154.0622			U13C Histidine	U13C Histidine	102	124	0.5 - 25	0.9998	0.01 - 25	0.9970
Homocysteine	3.20	C4H9NO2S	136.0427	134.0281	> 0.5 µM	> 1 µM	U13C Citrulline		183		0.5 - 10	0.9918		
Homoserine	3.88	C4H9NO3	120.0655	118.0510	0.08	0.06	U13C Homoserine	U13C Threonine	110	101	0.05 - 10	0.9986	0.5 - 25	0.9998
Hydroxyglutaric acid	4.05	C5H8O5	149.0445	147.0299		0.03		U13C Hydroxyglutaric acid		134			0.01 - 10	0.9858
IMP	4.18	C10H13N4O8P	349.0544	347.0398	0.10	0.2	U13C AMP	U13C IMP	86	98	0.05 - 10	0.9977	0.5 - 10	0.9979
Inosine	2.33	C10H12N4O5	269.0881	267.0735	0.05	0.03	U13C Adenosine	External	97	126	0.01 - 10	0.9987	0.01 - 10	0.9990
Isocitrate	6.22	C6H8O7	193.0343	191.0197		0.03	External	U13C Citrate		117			0.05 - 10	0.9944
Isoleucine+Leucine	2.34	C6H13NO2	132.1019	130.0874	0.05	0.04	U13C Isoleucine+Leucine	U13C Isoleucine+Leucine	110	108	0.02 - 50	0.9998	0.1 - 50	0.9997
Ketoisovalerate	1.05	C5H8O3	117.0546	115.0401		0.09		U13C Dihydroxyisovalerate		111			0.05 - 25	0.9983
Kynurenine	2.26	C10H12N2O3	209.0921	207.0775	0.3	0.2	U13C Citrulline	External	133	106	0.01 - 10	0.9976	0.05 - 10	0.9998
Lactate	1.64	C3H6O3	91.0390	89.0244		0.1		U13C Lactate		86			0.01 - 10	0.9715
Lysine	8.99	C6H14N2O2	147.1128	145.0983	0.07	0.02	U13C Lysine	U13C Lysine	103	110	0.5 - 25	0.9996	0.1 - 25	0.9992
Malate	4.53	C4H6O5	135.0288	133.0143		0.01		U13C Malate		116			0.05 - 25	0.9986

Compound	RT [min]	Formula	$[M+H]^+$	$[M-H]^-$	LOD pos [µM]	LOD neg [µM]	ISTD pos	ISTD neg	Recovery pos [%]	Recovery neg [%]	Linear range pos	R^2 pos	Linear range neg	R^2 neg
Mannitol	3.40	C6H14O6	183.0863	181.0718		0.01		U13C Mannitol		94			0.01 - 10	0.9962
Melatonine	1.02	C13H16N2O2	233.1285	231.1139	0.1	0.07	External	U13C Proline	104	105	0.01 - 1	0.9999	0.01 - 25	0.9969
Methionine	2.48	C5H11NO2S	150.0583	148.0438	0.03	0.03	U13C Methionine	U13C Methionine	94	98	0.01 - 25	0.9988	0.05 - 25	0.9973
Methionine sulfone	2.59	C5H11NO4S	182.0482	180.0336	0.02	0.02	U13C Methionine	U13C Methionine	94	124	0.05 - 10	0.9946	0.01 - 25	0.9995
Mevalonic acid	1.41	C6H12O4	149.0808	147.0663		0.03		U13C Mevalonic acid		116			0.01 - 10	0.9960
N4-Acetylcytidine	1.53	C11H15N3O6	286.1034	284.0888	0.09	0.05	U13C Adenosine	U13C AMP	87	113	0.01 - 25	0.9974	0.05 - 25	0.9986
N-Acetyl-Asp-Glu	5.38	C11H16N2O8	305.0979	303.0834	0.1		U13C Aspartate		76		0.01 - 25	0.9954	5 - 25	0.9955
N-Acetyl-L-aspartic acid	3.77	C6H9NO5	176.0554	174.0408	> 0.5 µM	0.07	U13C Aspartate	U13C N-Acetyl-L-aspartic acid		102	1 - 25	0.9961	0.01 - 25	0.9959
N-Acetyl-serine	1.98	C5H9NO4	148.0604	146.0459	0.1	0.03	U13C Serine	U13C Serine	111	141	0.05 - 10	0.9956	0.01 - 25	0.9935
NAD+	3.37	C21H27N7O14P2	664.1164	662.1018	0.07	0.06	U13C NAD+	U13C NAD+	111	108	0.05 - 25	0.9996	0.05 - 25	0.9995
NADH	3.27	C21H29N7O14P2	666.1321	664.1175		> 1 µM	U13C NADH	U13C NADH			5 - 25	0.9984	0.5 - 25	0.9983
NADP+	5.24	C21H28N7O17P3	744.0827	742.0682	0.06	0.1	U13C NADP+	U13C NADP+	106	104	0.01 - 25	0.9987	0.05 - 10	0.9994
NADPH	5.45	C21H30N7O17P3	746.0984	744.0838	0.3		U13C NADH		107		0.5 - 25	0.9941	1 - 25	0.7391
Nicotinamide	1.33	C6H6N2O	123.0553	121.0407	0.004		U13C Nicotinamide		99		0.05 - 25	0.9970	5 - 25	0.7943
Octopamine	7.26	C8H11NO2	154.0863	152.0717	0.08		U13C Citrulline		110		0.05 - 25	0.9981		
Ornithine	8.33	C5H12N2O2	133.0972	131.0826	0.08	0.03	U13C Ornithine	U13C Ornithine	127	110	0.01 - 10	0.9924	0.05 - 25	0.9993
Oxaloacetic acid	4.90	C4H4O5	133.0132	130.9986		> 1 µM		U13C Succinate					5 - 25	0.9985
Phenylalanine	1.99	C9H11NO2	166.0863	164.0717	0.02	0.02	U13C Phenylalanine	U13C Phenylalanine	103	97	0.05 - 25	0.9991	0.01 - 25	0.9990
Phosphocreatine	4.14	C4H10N3O5P	212.0431	210.0285	0.1	0.08	External		60	60	0.05 - 10	0.9852	0.1 - 25	0.9912
Proline	2.92	C5H9NO2	116.0706	114.0561	0.01	0.1	U13C Proline	U13C Proline	112	105	0.01 - 25	0.9993	0.5 - 25	0.9969
Propionyl-L-carnitine	1.79	C10H19NO4	218.1387	216.1241	0.03		External		100		0.01 - 10	0.9998		
Pseudouridine	2.69	C9H12N2O6	245.0768	243.0623		0.06		U13C Pseudouridine		97			0.01 - 10	0.9994
Pyruvate	1.40	C3H4O3	89.0233	87.0088		0.2		U13C Pyruvate		108			0.5 - 25	0.9995
Ribose	2.61	C5H10O5	151.0601	149.0456		0.3		U13C Ribose		87			0.5 - 10	0.9976
Pentose-phosphate	4.37	C5H11O8P	231.0264	229.0119	0.5	0.2	U13C F6P+G1P	U13C Hexose-P	113	122	0.2 - 20	0.9975	0.2 - 50	0.9976
S-Adenosyl-methionine	5.19	C15H22N6O5S	399.1445	397.1300	0.1		External		90		0.05 - 25	0.9950	1 - 25	0.9995
S-Adenosyl-homocysteine	3.24	C14H20N6O5S	385.1289	383.1143	0.1	0.04	U13C Adenosine	U13C AMP	114	106	0.01 - 25	0.9992	0.05 - 10	0.9855
Sarcosine	3.43	C3H7NO2	90.0550	88.0404	0.3	0.08	U13C Alanine	U13C Alanine	114	99	0.01 - 10	0.9981	0.5 - 25	0.9988
Sedoheptulose-7-phosphate	4.66	C7H15O10P	291.0476	289.0330		0.07		U13C Sedoheptulose-7-phosphate		102			0.5 - 10	0.9966
Seleno-methionine	2.43	C5H11NO2Se	198.0028	195.9882	0.03	0.03	U13C Methionine	U13C Methionine	99	100	0.01 - 10	0.9990	0.05 - 10	0.9993
Serine	4.22	C3H7NO3	106.0499	104.0353	0.05	0.2	U13C Serine	U13C Serine	104	105	0.01 - 25	0.9996	0.5 - 25	0.9939
Spermidine	n.d.	C7H19N3	146.1652	144.1506										
Spermine	n.d.	C10H26N4	203.2230	201.2085										

Compound	RT [min]	Formula	$[M+H]^+$	$[M-H]^-$	LOD pos [µM]	LOD neg [µM]	ISTD pos	ISTD neg	Recovery pos [%]	Recovery neg [%]	Linear range pos	R^2 pos	Linear range neg	R^2 neg
Succinate	4.01	C4H6O4	119.0339	117.0193		0.01		U13C Succinate		111			0.01 - 25	0.9996
Thiamine	n.d.	C12H17N4OS	266.1196	264.1050										
Threonine	3.55	C4H9NO3	120.0655	118.0510	0.06	0.1	U13C Threonine	U13C Threonine	93	101	0.1 - 25	0.9983	0.5 - 25	0.9999
Thymidine	1.32	C10H14N2O5	243.0976	241.0830	0.1	0.02	U13C Adenosine	U13C Adenine	92	111	0.5 - 5	0.9974	0.01 - 10	0.9877
Thymine	1.39	C5H6N2O2	127.0502	125.0357	0.4	0.01	U13C Adenosine	U13C Adenine		104			0.05 - 10	0.9884
TMP	2.80	C10H15N2O8P	323.0639	321.0493	0.07	0.03	U13C AMP	U13C AMP	96	108	0.05 - 25	0.9993	0.01 - 25	0.9987
Trehalose	4.40	C12H22O11	343.1235	341.1089		> 1 µM		U13C Trehalose					5 - 25	0.9982
Tryptophan	2.57	C11H12N2O2	205.0972	203.0826	0.02	0.06	U13C Tryptophan	U13C Tryptophan	105	110	0.01 - 25	0.9993	0.05 - 25	0.9991
TTP	4.92	C10H17N2O14P3	482.9965	480.9820	0.2	0.02	U13C AMP	U13C ATP	86	111	0.5 - 25	0.9979	0.1 - 25	0.9998
Tyrosine	3.08	C9H11NO3	182.0812	180.0666	0.07	0.07	U13C Tyrosine	U13C Tyrosine	101	103	0.01 - 25	0.9993	0.05 - 25	0.9996
UDP	5.11	C9H14N2O12P2	405.0095	402.9949	0.1	0.03	U13C ADP	U13C UDP	108	114	0.1 - 25	0.9997	0.05 - 25	0.9985
UMP	3.95	C9H13N2O9P	325.0431	323.0286	0.1	0.2	U13C AMP	U13C UMP	93	103	0.5 - 25	0.9986	0.1 - 25	0.9996
Uracil	1.69	C4H4N2O2	113.0346	111.0200	> 1 µM	0.09		U13C Adenine		113			0.1 - 10	0.9928
Uridine	2.03	C9H12N2O6	245.0768	243.0623		0.05		U13C Pseudouridine		80			0.01 - 10	0.9821
UTP	5.86	C9H15N2O15P3	484.9758	482.9613	0.1	0.04	U13C ATP	U13C UTP	112	103	0.5 - 25	0.9990	0.1 - 25	0.9993
Valine	2.80	C5H11NO2	118.0863	116.0717	> 1 µM	0.08	U13C Valine	U13C Valine		107	0.01 - 10	0.9867	0.05 - 25	0.9985
Xanthine	2.35	C5H4N4O2	153.0407	151.0262		0.06		U13C Adenine		148			0.01 - 10	0.9955
Xylose	3.22	C5H10O5	151.0601	149.0456		> 0.5 µM		External		79			1 - 25	0.9920

Table S2. Isomer separation achieved with the dual setup (11 min gradient on pHILIC column).

Isomers	Separation (Elution order where possible to give)
Citrate/isocitrate	Baseline separated (citrate – isocitrate)
Sarcosine/alanine	Baseline separated (sarcosine – alanine)
Leucine/isoleucine	Coelute (leucine – isoleucine)
Betaine/valine	Not baseline separated (betaine – valine), betaine much better ionized in positive, valine in negative ESI
Glutamate/N-acetylserine	Baseline separated (N-acetylserine – glutamate)
Ribose-5P/ribulose-5P	Coelute
Hexose-phosphates	F6P and G6P baseline separated, F6P and G1P coelute (F6P&G1P – G6P)
Hexoses	Coelute (fructose – mannose – galactose – glucose)
AMP	Baseline separated (3'AMP – 5'AMP)
ATP/dGTP	Baseline separated (ATP – dGTP)

Table S3. List of lipids, retention times, sum formulas, detected *m/z* values of adducts, limits of detection for an injection volume of 5 µL, recoveries of metabolite concentrations in a 1 µM QC sample (giving the trueness bias), linear calibration ranges and corresponding correlation coefficients for both positive and negative electrospray ionization based on external calibration.

Compound	RT [min]	Formula	m/z pos	Adduct pos	m/z neg	Adduct neg	LOD pos [µM]	LOD neg [µM]	Recovery pos	Recovery neg	Linear range pos	R^2 pos	Linear range neg	R^2 neg
AcCa 18:1	16.40	C25H47NO4	426.3578	M+H	470.3487	M+HCOO	0.17	0.13	101%	87%	0.01 - 5	0.9980	0.01 - 10	0.9976
AcCa 6:0	11.42	C13H25NO4	260.1856	M+H	304.1766	M+HCOO		0.16		89%			0.05 - 10	0.9993
CE 16:0	30.82	C43H76O2	642.6184	M+NH4			> 1 µM							
CE 18:0	31.12	C45H80O2	670.6497	M+NH4			> 1 µM							
CE 18:2	30.46	C45H76O2	666.6184	M+NH4			> 1 µM							
Cer d34:1	27.67	C34H67NO3	538.5194	M+H	582.5103	M+Cl	0.12	0.27	118%	90%	0.01 - 1	0.9647	0.01 - 1	0.9812
Cer d36:1	28.23	C36H71NO3	566.5503	M+H	610.5412	M+Cl	0.04	0.12	81%	92%	0.01 - 5	0.9765	0.01 - 1	0.9886
Cholesterol	27.07	C27H46O	369.3516	M+H-H2O			> 1 µM				5 - 25	0.9388		
CL 64:4	29.14	C73H134O17P2	1362.9435	M+NH4	1343.9024	M-H	0.04	0.06	60%	78%	0.05 - 25	0.9936	0.05 - 25	0.9988
CL 72:4	30.05	C81H150O17P2	1475.0687	M+NH4	1456.0276	M-H	0.02	0.96	70%	53%	0.5 - 25	0.9679	0.5 - 25	0.9659
DG 32:0	28.50	C35H68O5	586.5405	M+NH4	603.4761	M+Cl	0.06		70%		0.1 - 10	0.9860		
DG 34:1	28.51	C37H70O5	612.5558	M+NH4	639.5202	M+Cl	0.03		70%		0.05 - 10	0.9831	5 - 25	0.9796
DG 36:2	28.53	C39H72O5	638.5718	M+NH4	665.5362	M+Cl	0.07		72%		0.01 - 10	0.9844		
Ergosterol	25.99	C28H44O	397.3465	M+H			0.04		93%		0.1 - 10	0.9943		

Compound	RT [min]	Formula	m/z pos	Adduct pos	m/z neg	Adduct neg	LOD pos [µM]	LOD neg [µM]	Recovery pos	Recovery neg	Linear range pos	R^2 pos	Linear range neg	R^2 neg
FA 16:0	21.40	C16H32O2			255.2327	M-H		> 1 µM					1 - 25	0.9927
FA 18:0	23.62	C18H36O2			283.2647	M-H		> 1 µM					1 - 25	0.9976
FA 20:1	23.78	C20H38O2			309.2797	M-H		> 1 µM					5 - 25	0.9911
FA 22:0	27.05	C22H44O2			339.3267	M-H		> 1 µM					5 - 25	0.9497
Hex2Cer d34:1	26.13	C46H87NO13	862.6253	M+H	906.6162	M+Cl	0.03	0.81	75%	91%	0.01 - 10	0.9893	0.5 - 5	0.9982
HexCer d34:1	26.64	C40H77NO8	700.5723	M+H	734.5344	M+HCOO	0.04	0.09	65%	96%	0.02 - 20	0.9814	0.02 - 10	0.9917
LPC 16:0	16.15	C24H50NO7P	496.3393	M+H	540.3302	M+Cl	0.15	0.10	101%	106%	0.01 - 10	0.9928	0.1 - 10	0.9974
LPC 18:0/0:0	18.83	C26H54NO7P	524.3711	M+H	568.3620	M+Cl	0.04	0.18	77%	95%	0.01 - 10	0.9924	0.5 - 25	0.9990
MG 16:0	19.53	C19H38O4	331.2843	M+H	375.2752	M+Cl	0.77		113%		1 - 25	0.9997	5 - 25	0.9563
PA 34:1	27.60	C37H71O8P	675.4959	M+H	673.4814	M-H	0.08	0.12	91%	82%	0.5 - 10	0.9930	0.05 - 25	0.9950
PA 36:2	27.61	C39H73O8P	701.5116	M+H	699.4970	M-H	0.05	0.09	84%	89%	0.5 - 10	0.9922	0.05 - 25	0.9958
PC 34:0	29.01	C42H84NO8P	762.6007	M+H	806.5917	M+HCOO	0.14	0.17	131%	86%	0.01 - 10	0.9899	0.1 - 5	0.9921
PC 34:1	27.88	C42H82NO8P	760.5851	M+H	804.5760	M+HCOO	0.02	0.04	93%	100%	0.05 - 10	0.9924	0.05 - 10	0.9940
PC 34:2	27.03	C42H80NO8P	758.5693	M+H	802.5604	M+HCOO	0.03	0.05	91%	107%	0.05 - 10	0.9911	0.05 - 10	0.9926
PC 36:2	28.21	C44H84NO8P	786.6007	M+H	820.5629	M+HCOO	0.18	0.27	93%	113%	0.03 - 30	0.9893	0.15 - 75	0.9874
PE 34:1	27.62	C39H76NO8P	718.5383	M+H	716.5237	M-H	0.11	0.20	103%	113%	0.01 - 10	0.9806	0.01 - 10	0.9990
PE 36:2	27.68	C41H78NO8P	744.5543	M+H	742.5397	M-H	0.10	0.15	105%	107%	0.01 - 10	0.9881	0.01 - 10	0.9979
PG 34:1	26.60	C40H77O10P	749.5327	M+H	747.5182	M-H	0.05	0.05	83%	105%	0.05 - 25	0.9967	0.01 - 25	0.9955
PG 36:2	26.69	C42H79O10P	775.5484	M+H	773.5338	M-H	0.07	0.04	79%	100%	0.05 - 10	0.9942	0.01 - 25	0.9971
PS 34:1	27.07	C40H76NO10P	762.5280	M+H	760.5134	M-H	0.01	0.03	79%	91%	0.05 - 10	0.9925	0.1 - 10	0.9976
PS 36:2	27.07	C42H78NO10P	788.5436	M+H	786.5291	M-H	0.01	0.05	78%	90%	0.05 - 10	0.9899	0.05 - 25	0.9988
SM d36:2	26.78	C41H81N2O6P	729.5905	M+H	773.5814	M+HCOO	0.02	0.10	62%	99%	0.01 - 10	0.9793	0.05 - 10	0.9995
SPH d18:1	14.58	C18H37NO2	300.2897	M+H			0.10		68%		0.05 - 25	0.9926		
TG 12:0	12.99	C15H26O6	320.2068	M+NH4			0.12		152%		0.01 - 10	0.9721		
TG 18:0	18.72	C21H38O6	404.3008	M+NH4			0.06		101%		0.01 - 10	0.9995		
TG 24:0	24.16	C27H50O6	488.3948	M+NH4			0.02		80%		0.01 - 10	0.9849		
TG 30:0	27.68	C33H62O6	572.4885	M+NH4			0.09		95%		0.01 - 5	0.9830		
TG 36:0	28.85	C39H74O6	656.5824	M+NH4			0.14		86%		0.01 - 10	0.9946		
TG 42:0	29.39	C45H86O6	740.6763	M+NH4			0.16		92%		0.01 - 5	0.9957		
TG 48:0	29.88	C51H98O6	824.7701	M+NH4			> 1 µM							
TG 48:3	29.38	C51H92O6	818.7232	M+NH4			0.10		58%		0.01 - 25	0.9744		
TG 54:0	30.46	C57H110O6	908.8641	M+NH4			> 1 µM							
TG 54:3	29.86	C57H104O6	902.8171	M+NH4			> 1 µM							
TG 54:6	29.43	C57H98O6	896.7702	M+NH4			0.07		52%		0.01 - 25	0.9680		
TG 54:9	29.07	C57H92O6	890.7232	M+NH4			0.03		58%		0.01 - 25	0.9822		
TG 60:3	30.38	C63H116O6	986.9110	M+NH4			> 1 µM							
TG 66:3	31.02	C69H128O6	1071.0049	M+NH4			> 1 µM							
TG 6:0	11.58	C9H14O6	236.1128	M+NH4			0.03		108%		0.5 - 25	0.9997		

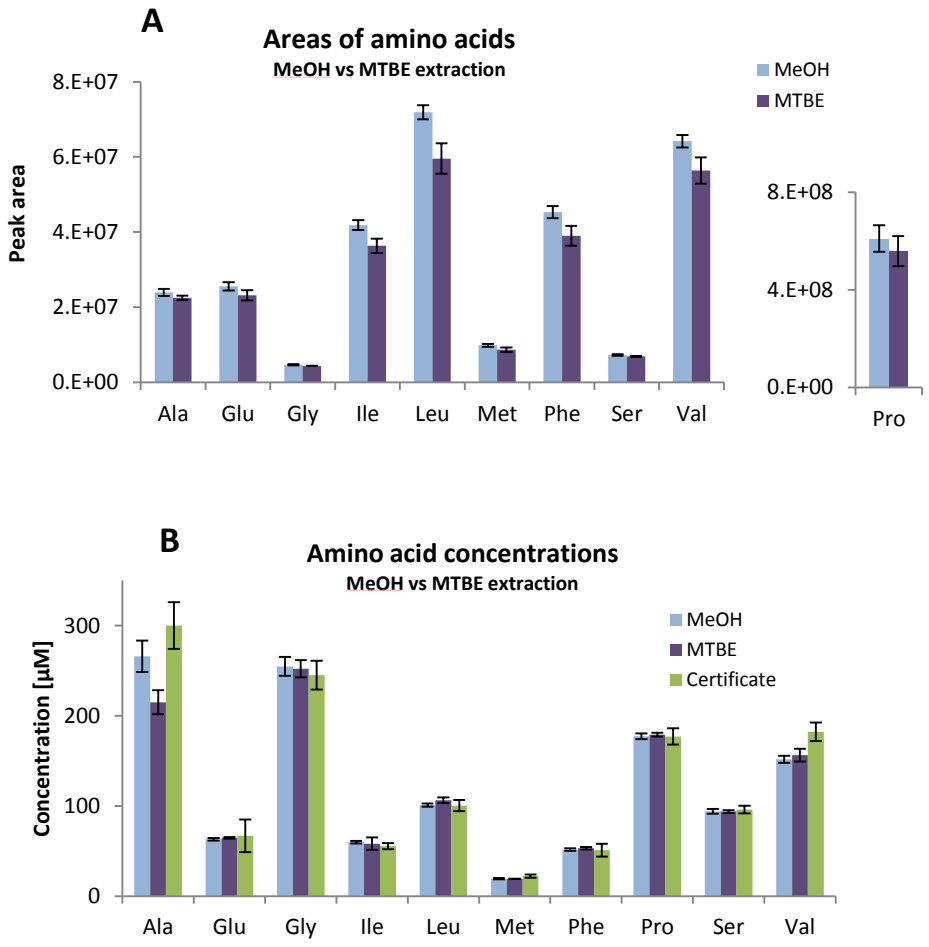

Fig. S1: Peak areas (A) and calculated amino acid concentrations based on internal standardization with fully ^{13}C labeled yeast extract (B) in human plasma SRM 1950 from MeOH extraction compared to biphasic MTBE extraction. Data measured with a Trinity P2 column.

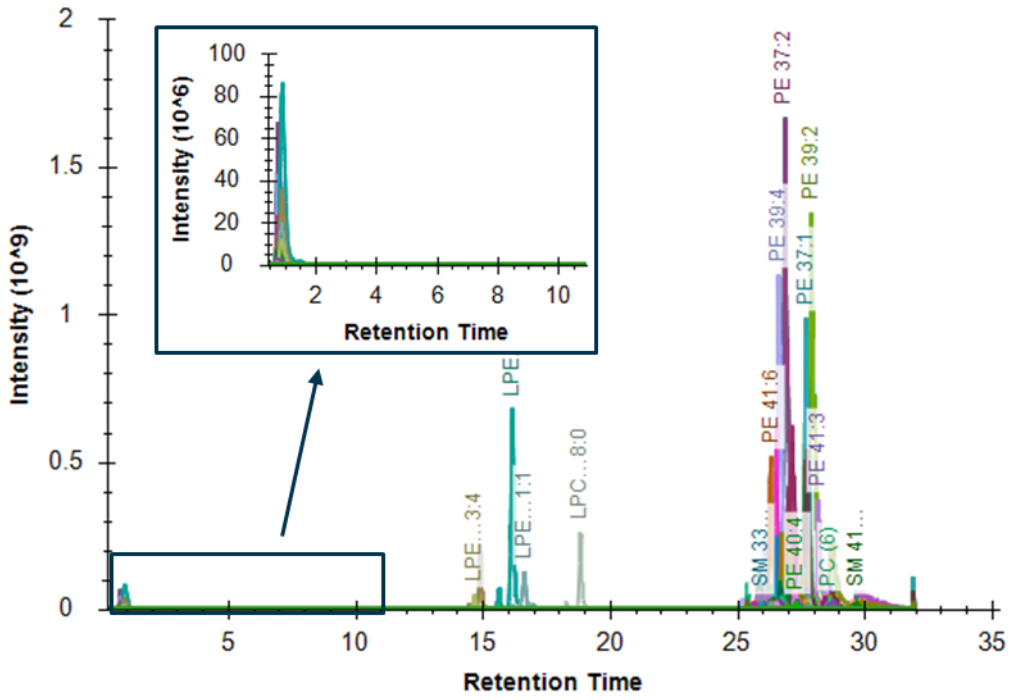

Fig. S2: Extracted ion chromatograms of lipids eluting from the dual HILIC-RP separation. Lipids present in the MTBE extract and injected onto the RP column eluted between 11 and 32 min. At 1 min, lipids that were extracted by MeOH and hence injected onto the HILIC column for metabolite separation can be seen. Since they all eluted in the void volume, no interference with retained metabolites was observed.

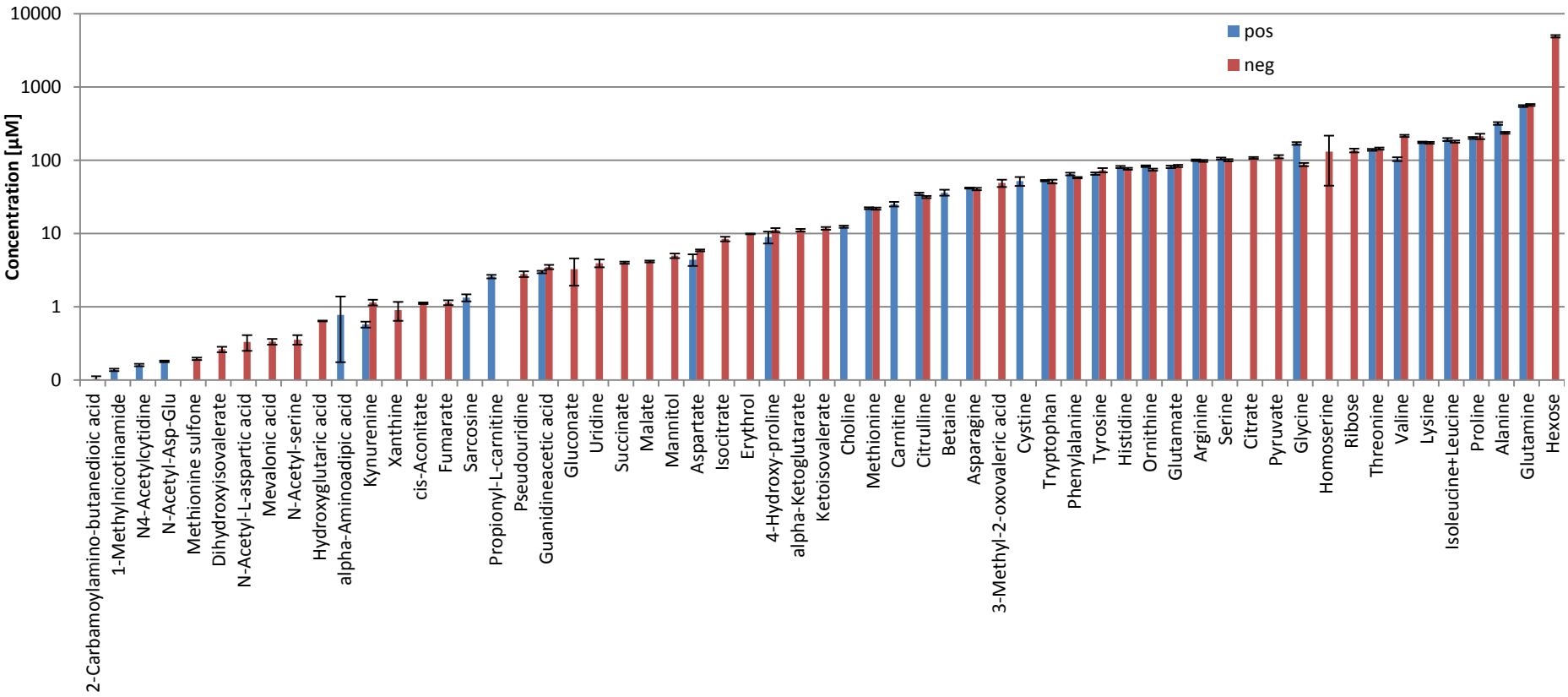

Fig. S3: Results of metabolites that were quantified in "SRM 1950 – Metabolites in human plasma" based on external calibration with internal standardization. Concentration ranges over more than four orders of magnitude were observed. Data from positive (blue) and negative (red) electrospray ionization measured in polarity switching mode are presented with standard deviations of N=4 extraction replicates. Logarithmic scale for concentration. Among the quantified metabolites only for amino acids certified values are available (shown in Figure 3).

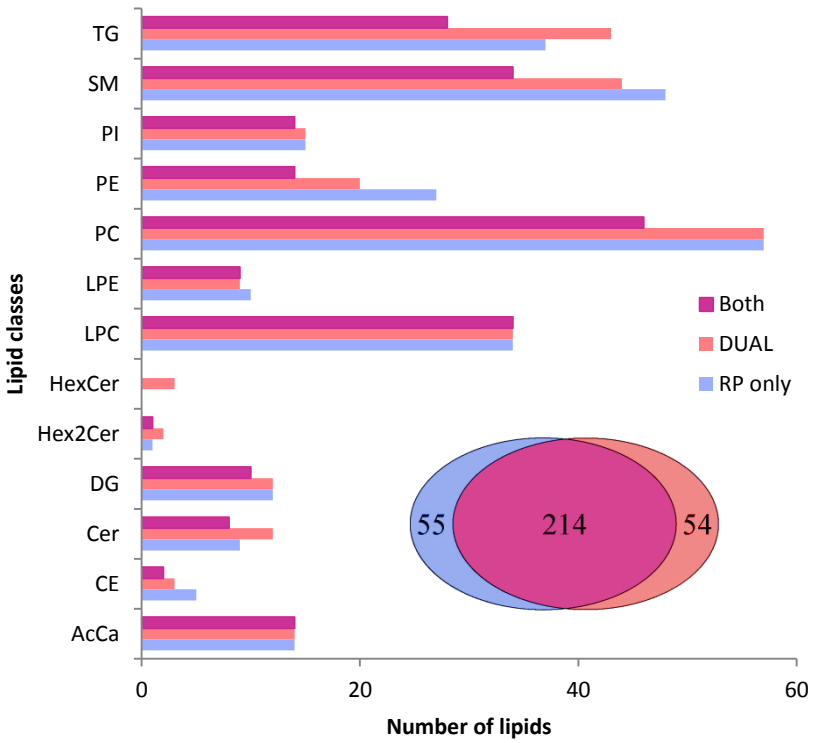

Fig. S4: Comparison of lipid identification performed on data from the DUAL setup compared to RP only runs. The number of identified lipids separated into lipid classes is shown for the two separations. N=4 extraction replicates.

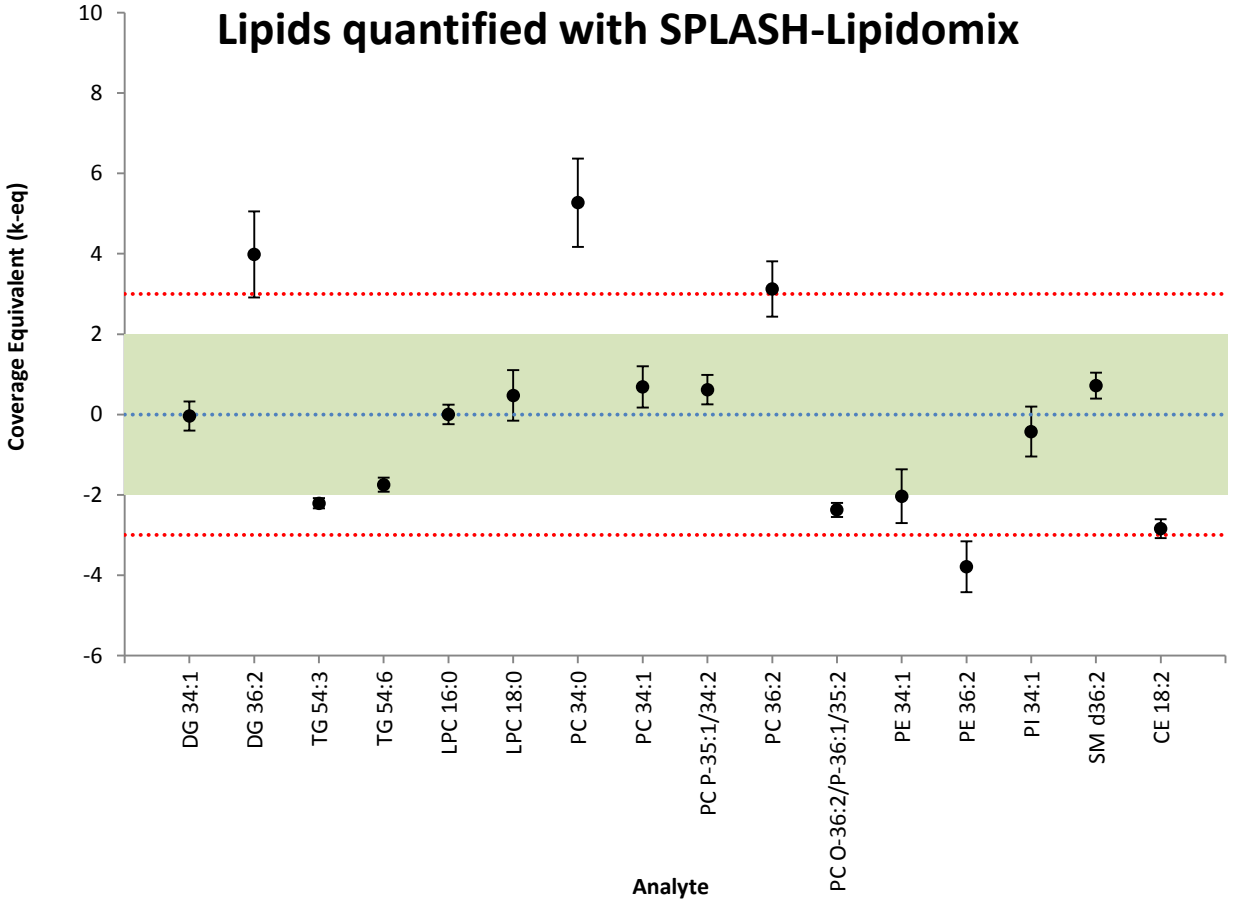

Fig. S5: Accuracy assessment of lipid quantification for SRM 1950 human plasma with SPLASH-Lipidomix. Values are presented as normalized coverage equivalents at the mean (dots) and standard deviation (error bars) of measurements, overlaid onto the consensus mean value (blue line) and uncertainty (95% coverage-green region, 99% coverage-red region). The figure was prepared using the LipidQC tool.[1]

References

(1) Ulmer, C. Z.; Ragland, J. M.; Koelmel, J. P.; Heckert, A.; Jones, C. M.; Garrett, T. J.; Yost, R. A.; Bowden, J. A. LipidQC: Method Validation Tool for Visual Comparison to SRM 1950 Using NIST Interlaboratory Comparison Exercise Lipid Consensus Mean Estimate Values. *Anal. Chem.* **2017**, *89* (24), 13069–13073.