Supplementary Materials for Multi-Dimensional Liquid Chromatography of Pulse Triacylglycerols with Triple Parallel Mass Spectrometry

William Byrdwell and Hari Kiran Kotapati

Supplementary Table S1. Concentrations of fat-soluble vitamins (FSVs) and triacylglycerols (TAGs) in final calibration standard mixtures.

	Std. 1	[1,3 Isomers]		Std. 2	[1,2 Isomers]		Std. 3	[1,3 Isomers]		Std. 4	[1,2 Isomers]		Std. 5	[1,3 Isomers]	
	μg/mL	nMol/mL	Wt. %	μg/mL	nMol/mL	Wt. %	μg/mL	nMol/mL	Wt. %	μg/mL	nMol/mL	Wt. %	μg/mL	nMol/mL	Wt. %
Vit. K2	2.00100	4.50021	80.29%	2.00100	4.50021	67.07%	2.00100	4.50021	44.89%	2.00100	4.50021	28.94%	2.00100	4.50021	16.92%
Vitamin D2	0.05002	0.12609	2.25%	0.10003	0.25219	3.76%	0.25008	0.63047	6.29%	0.50015	1.26094	8.11%	1.00030	2.52189	9.48%
Vitamin D3	0.05001	0.13001	2.32%	0.10001	0.26001	3.88%	0.25003	0.65003	6.48%	0.50005	1.30006	8.36%	1.00010	2.60012	9.78%
α-Tocopherol	0.05000	0.11609	2.07%	0.10000	0.23218	3.46%	0.25000	0.58044	5.79%	0.50000	1.16089	7.47%	1.00000	2.32178	8.73%
δ-Tocopherol	0.05000	0.12418	2.22%	0.10000	0.24835	3.70%	0.25000	0.62088	6.19%	0.50000	1.24177	7.99%	1.00000	2.48354	9.34%
γ-Tocopherol	0.05000	0.12000	2.14%	0.10000	0.23999	3.58%	0.25000	0.59998	5.99%	0.50000	1.19997	7.72%	1.00000	2.39993	9.02%
α-Toco Acetate	0.05000	0.10714	1.91%	0.10000	0.21427	3.19%	0.25000	0.53568	5.34%	0.50000	1.07137	6.89%	1.00000	2.14273	8.06%
Retinol	0.05012	0.17497	3.12%	0.10024	0.34994	5.22%	0.25060	0.87485	8.73%	0.50120	1.74969	11.25%	1.00240	3.49938	13.16%
Retinyl Palmitate	0.05000	0.09526	1.70%	0.10000	0.19053	2.84%	0.25000	0.47632	4.75%	0.50000	0.95264	6.13%	1.00000	1.90527	7.16%
Phylloquinone	0.05007	0.11110	1.98%	0.10014	0.22219	3.31%	0.25035	0.55548	5.54%	0.50070	1.11095	7.15%	1.00140	2.22191	8.35%
7,7,7	8.835	20.614	8.47%	8.835	20.614	5.21%	8.835	20.614	2.84%	8.835	20.614	1.25%	8.835	20.614	0.62%
11,11,11	12.305	20.614	8.47%	12.305	20.614	5.21%	12.305	20.614	2.84%	12.305	20.614	1.25%	12.305	20.614	0.62%
15,15,15	15.770	20.608	8.47%	15.770	20.608	5.20%	15.770	20.608	2.84%	15.770	20.608	1.25%	15.770	20.608	0.62%
19,19,19	19.235	20.604	8.47%	19.235	20.604	5.20%	19.235	20.604	2.84%	19.235	20.604	1.25%	19.235	20.604	0.62%
9,9,9	2.114	4.123	1.69%	4.230	8.249	2.08%	8.456	16.491	2.27%	21.150	41.247	2.50%	42.280	82.456	2.50%
13,13,13	2.807	4.121	1.69%	5.612	8.240	2.08%	11.228	16.486	2.27%	28.060	41.199	2.50%	56.140	82.428	2.50%
17,17,17	3.500	4.121	1.69%	7.000	8.241	2.08%	14.000	16.482	2.27%	35.000	41.206	2.50%	70.000	82.411	2.50%
21,21,21	4.195	4.122	1.69%	8.390	8.244	2.08%	16.780	16.488	2.27%	41.950	41.220	2.50%	83.900	82.439	2.50%
CaMCa/CaCaM	2.510	4.108	1.69%	5.180	8.479	2.14%	10.040	16.434	2.26%	25.900	42.393	2.57%	50.200	82.168	2.49%
LaMLa/LaLaM	5.010	7.511	3.09%	5.100	7.646	1.93%	20.040	30.043	4.14%	25.500	38.228	2.32%	100.200	150.213	4.55%
LaPLa/LaLaP	5.020	7.222	2.97%	10.060	14.473	3.65%	20.080	28.888	3.98%	50.300	72.363	4.38%	100.400	144.438	4.37%
LaOLa/LaLaO	5.020	6.961	2.86%	10.020	13.895	3.51%	20.080	27.845	3.83%	50.100	69.473	4.21%	100.400	139.223	4.22%
PLP/PPL	2.730	3.284	1.35%	5.160	6.207	1.57%	10.920	13.135	1.81%	25.800	31.034	1.88%	54.600	65.677	1.99%
POP/PPO	2.660	3.192	1.31%	5.260	6.312	1.59%	10.640	12.768	1.76%	26.300	31.559	1.91%	53.200	63.838	1.93%
LPL/LLP	2.510	2.934	1.21%	5.020	5.869	1.48%	10.040	11.738	1.62%	25.100	29.344	1.78%	50.200	58.689	1.78%
OPO/OOP	2.620	3.049	1.25%	5.040	5.865	1.48%	10.480	12.195	1.68%	25.200	29.323	1.78%	52.400	60.973	1.85%
LOL/LLO	2.520	2.859	1.17%	5.680	6.444	1.63%	10.080	11.436	1.57%	28.400	32.222	1.95%	50.400	57.182	1.73%
OLO/OOL	2.600	2.943	1.21%	5.140	5.818	1.47%	10.400	11.773	1.62%	25.700	29.092	1.76%	52.000	58.863	1.78%
OSO/OOS	2.540	2.862	1.18%	5.120	5.769	1.46%	10.160	11.449	1.58%	25.600	28.847	1.75%	50.800	57.243	1.73%

SOS/SSO	2.570	2.889	1.19%	5.240	5.891	1.49%	10.280	11.558	1.59%	26.200	29.456	1.78%	51.400	57.788	1.75%
OAO/OOA	2.510	2.742	1.13%	5.020	5.483	1.38%	10.040	10.967	1.51%	25.100	27.417	1.66%	50.200	54.833	1.66%
ODhO/OODh	2.730	2.931	1.20%	5.040	5.411	1.37%	10.920	11.724	1.61%	25.200	27.054	1.64%	54.600	58.618	1.78%
POLa/OLaP	2.720	3.500	1.44%	5.080	6.536	1.65%	10.880	13.998	1.93%	25.400	32.679	1.98%	54.400	69.990	2.12%
PLO/OPL	2.740	3.196	1.31%	5.360	6.252	1.58%	10.960	12.783	1.76%	26.800	31.258	1.89%	54.800	63.916	1.94%
POS/SPO	2.580	2.995	1.23%	5.160	5.990	1.51%	10.320	11.980	1.65%	25.800	29.951	1.81%	51.600	59.902	1.81%
SLO/SOL	2.680	3.027	1.24%	5.060	5.715	1.44%	10.720	12.107	1.67%	25.300	28.574	1.73%	53.600	60.536	1.83%
AcAcAc	2.000	9.166	3.77%	4.000	18.332	4.63%	8.000	36.663	5.05%	20.000	91.658	5.55%	40.000	183.315	5.55%
BuBuBu	2.000	6.615	2.72%	4.000	13.229	3.34%	8.000	26.458	3.64%	20.000	66.146	4.01%	40.000	132.291	4.01%
CoCoCo	3.667	9.486	3.90%	7.333	18.973	4.79%	14.667	37.945	5.22%	36.667	94.863	5.75%	73.333	189.726	5.75%
CyCyCy	5.667	12.039	4.95%	11.333	24.079	6.08%	22.667	48.157	6.63%	56.667	120.393	7.30%	113.333	240.786	7.29%
CaCaCa	5.667	10.213	4.20%	11.333	20.426	5.16%	22.667	40.853	5.62%	56.667	102.131	6.19%	113.333	204.263	6.19%
LaLaLa	3.667	5.738	2.36%	7.333	11.476	2.90%	14.667	22.953	3.16%	36.667	57.381	3.48%	73.333	114.763	3.48%
MMM	3.667	5.070	2.08%	7.333	10.141	2.56%	14.667	20.281	2.79%	36.667	50.703	3.07%	73.333	101.407	3.07%
PPP	2.000	2.477	1.02%	4.000	4.955	1.25%	8.000	9.909	1.36%	20.000	24.773	1.50%	40.000	49.547	1.50%
SSS	1.667	1.870	0.77%	3.333	3.739	0.94%	6.667	7.478	1.03%	16.667	18.696	1.13%	33.333	37.391	1.13%
OOO	7.002	7.908	3.25%	14.008	15.821	4.00%	28.008	31.632	4.36%	70.040	79.103	4.79%	140.040	158.160	4.79%
LLL	5.000	5.686	2.34%	10.000	11.372	2.87%	20.000	22.743	3.13%	50.000	56.858	3.45%	100.000	113.716	3.44%
TAG Sum (μg/mL)	165.033	243.399	100.00%	268.125	396.008	100.00%	491.697	726.277	100.00%	1116.045	1650.284	100.00%	2233.905	3301.629	100.00%
FSV Sum (μg/mL)	2.451	5.605	100.00%	2.901	6.710	100.00%	4.252	10.024	100.00%	6.503	15.548	100.00%	11.005	26.597	100.00%

Supplementary Table S2. Solvent gradient for the first dimension, ^{1}D, of three dimensions of separation.

	Ch. A	Ch. D
Time	%MeOH	%DCM
0	75	25
5	75	25
25	65	35
40	62.5	37.5
52	45	55
62.5	20	80
64	10	90
65	75	25
75	75	25

Supplementary Table S3. Gradient program for first second dimension, ^{2}D(1), as it should be entered in the Agilent OpenLab Chemstation 2D-LC workstation software program.

	^{1}D Time:									
^{2}D Time	0	9.55	11.46	21.01	24.83	26.74	30.56	32.47	44.12	74.49
0	0	0	25	0	45	35	25	5	0	0
0.3	0	0	25	0	45	35	25	5	0	0
1.3	5	5	40	15	65	55	45	37	5	5
1.6	5	5	45	15	70	60	50	42	5	5
1.7	0	0	25	0	45	35	25	5	0	0

Supplementary Table S4. Solvent program gradient for the second second dimension, ^{2}D(2), UHPLC separation on a Ag^{+} ion column.

Time	Ch. A %MeOH	Ch. B %ACN	Ch. D %DCM	Flow Rate mL/min
0	0	100	0	1.00
5	0	100	0	1.00
10	13.91	75.09	11	1.00
20	42.1	44.9	13	1.00
21	44.94	41.86	13.2	1.00
25	56.39	29.61	14	1.00
29	42.98	42.09	14.93	1.10
30	39.4	45.3	15.3	1.05
34	24.41	58.59	17	0.90
35	20.5	62	17.5	1.00
39	4.17	75.13	20.7	0.90
40	0	78.6	21.4	0.90
44	0	74	26	0.90
45	0	73	27	1.15
49	0	68	32	1.10
50	0	67	33	1.10
55	0	58	42	1.00
56	0	56	44	1.00
60	0	45	55	1.00
64	0	32	68	1.00
65	2	28	70	1.00
69	15	10	75	1.00
70	0	100	0	1.00
75	0	100	0	1.00

Supplementary Table S5. Critical Ratio 2, or $[AA]^+/[AB]^+$, from triacylglycerol (TAG) regioisomer standards by electrospray ionization (ESI) high-resolution, accurate-mass (HRAM) mass spectrometry (MS) using LipidSearch and atmospheric pressure chemical ionization (APCI)-MS using QuanBrowser. Sorted by ESI-MS 1,3-isomer values.

TAG[a]	ESI-MS 1,3-Isomer	SD	ESI-MS 1,2-Isomer	SD	APCI-MS 1,3-Isomer	SD	APCI-MS 1,2-Isomer	SD
OPO/OOP	0.29	0.01	0.52	0.03	0.51	0.04	0.78	0.06
OSO/OOS	0.31	0.01	0.58	0.02	0.18	0.02	0.36	0.03
LPL/LLP	0.33	0.01	0.54	0.05	0.39	0.06	0.98	0.07
LaMLa/LaLaM	0.33	0.01	0.63	0.02	0.19	0.02	0.59	0.01
OAO/OOA	0.33	0.01	0.60	0.02	0.22	0.01	0.71	0.05
LaPLa/LaLaP	0.35	0.01	0.71	0.03	0.38	0.05	0.99	0.08
SOS/SSO	0.35	0.01	0.64	0.03	0.23	0.01	0.68	0.05
OLO/OOL	0.36	0.01	0.57	0.02	0.22	0.02	0.69	0.12
CaMCa/CaCaM	0.37	0.02	0.79	0.06	0.26	0.02	1.02	0.08
POP/PPO	0.37	0.01	0.68	0.02	0.18	0.02	0.59	0.06
LOL/LLO	0.38	0.03	0.63	0.02	0.41	0.02	1.29	0.12
PLP/PPL	0.39	0.03	0.71	0.02	0.21	0.02	0.52	0.07
LaOLa/LaLaO	0.41	0.02	0.82	0.03	0.27	0.02	1.08	0.07
ODhO/OODh	0.56	0.02	0.40	0.03	4.97	0.23	11.43	1.74
ABC/ABC	([AC]/([AB]+[BC]))		([AC]/([AB]+[BC]))		([AC]/([AB]+[BC]))		([AC]/([AB]+[BC]))	
POLa/OLaP	0.40	0.01	0.24	0.02	0.26	0.02	0.18	0.01
PLO/OPL	0.37	0.01	0.30	0.02	0.13	0.01	0.45	0.03
POS/SPO	0.36	0.01	0.31	0.01	0.17	0.01	0.25	0.02
SLO/SOL	0.36	0.03	0.33	0.02	0.20	0.04	0.70	0.07
	[BC]/[AB]	SD	[BC]/[AB]	SD	[BC]/[AB]	SD	[BC]/[AB]	SD
POLa/OLaP	1.30	0.10	1.89	0.12	0.92	0.05	1.49	0.05
PLO/OPL	0.95	0.05	0.96	0.03	1.49	0.09	1.97	0.31
POS/SPO	0.96	0.05	0.93	0.02	1.05	0.05	1.49	0.08
SLO/SOL	0.97	0.04	0.90	0.04	0.82	0.09	1.11	0.08

[a]Fatty acid abbreviations in Materials and Methods.

Supplementary Table S6. Quantification of regioisomers as %AAB based on Critical Ratio 2, $[AA]^+/[AB]^+$, for triacylglycerols (TAGs). See Critical Ratio 2 values in Supplementary Table 2. Part 1: APCI-MS; Part 2: ESI-MS using LipidSearch (LS); Part 3: ESI-MS using QuanBrowser (QB). Abbreviations in Materials and Methods.

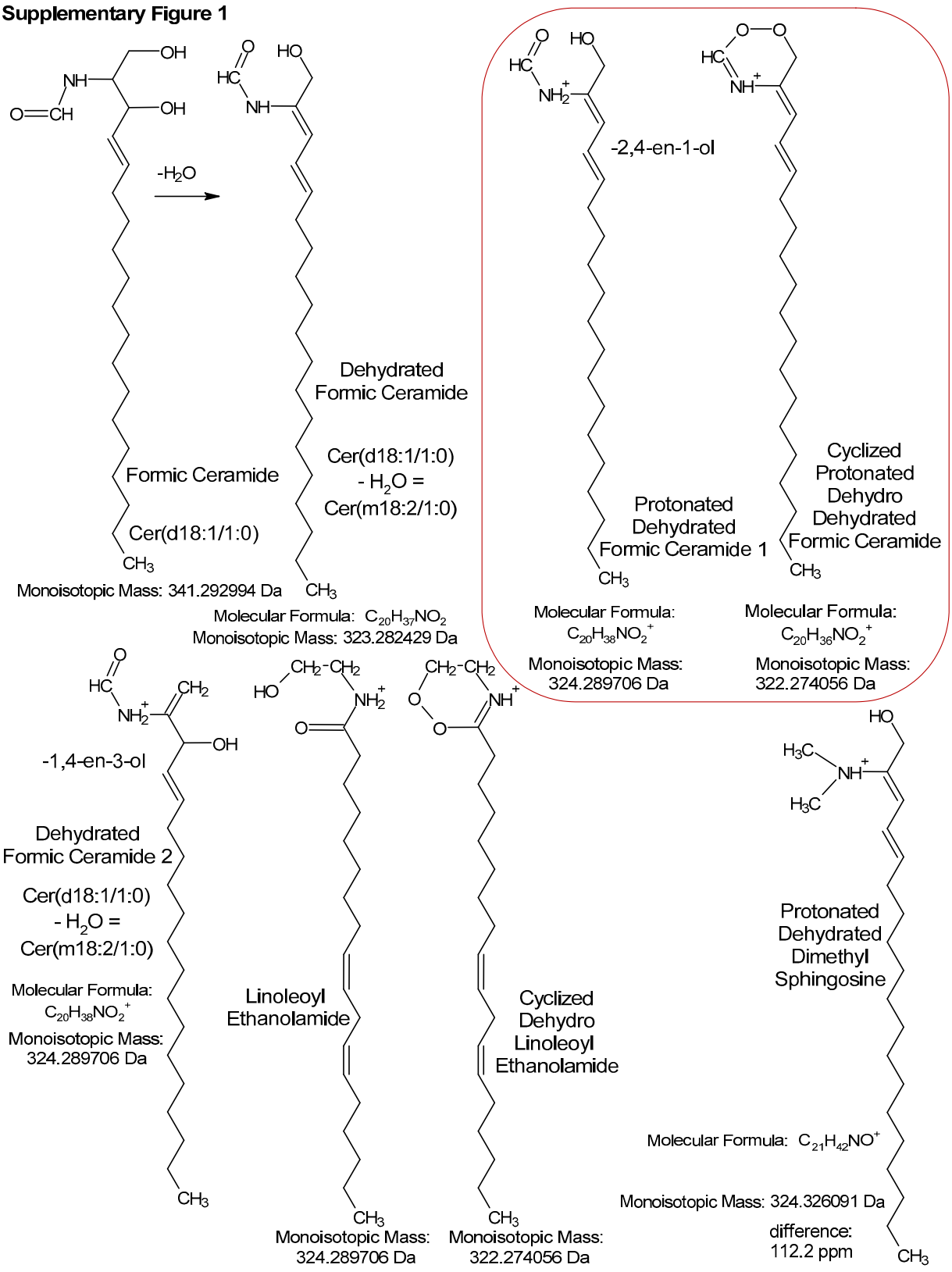
[1]D: APCI-MS			Pinto Beans	Navy Beans	Cranberry Beans	Black Beans	Black-Eyed Peas	Baby Lima Beans	Butter Beans	Lentils	Green Split Peas	Garbanzo Beans
TAG	RT	SD	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB
LOL/**LLO**	33.40	± 0.37	94.6%	76.2%	78.1%	84.4%	100.0%	69.9%	80.2%	64.7%	67.8%	75.7%
LPL/**LLP**	34.18	± 0.43	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
OLO/**OOL**	36.79	± 0.45	80.8%	40.6%	100.0%	51.0%	100.0%	84.1%	100.0%	73.9%	77.8%	43.8%
PLP/PPL	38.71	± 0.57	0.0%	4.6%	0.0%	0.3%	4.6%	4.2%	4.7%	3.7%	0.6%	0.0%
OPO/**OOP**	41.70	± 0.62	100.0%	58.9%	100.0%	100.0%	100.0%	100.0%	100.0%	95.1%	100.0%	78.0%
POP/PPO	42.91	± 0.70	69.2%	26.3%	22.8%	4.8%	4.5%	9.7%	11.7%	7.1%	1.6%	1.6%
OSO/**OOS**[a]	46.28	± 0.76	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

[2]D(2): ESI-MS (LS)			Pinto Beans	Navy Beans	Cranberry Beans	Black Beans	Black-Eyed Peas	Baby Lima Beans	Butter Beans	Lentils	Green Split Peas	Garbanzo Beans
TAG	RT	SD	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB
LOL/**LLO**	41.64	0.47	71.9%	76.4%	75.3%	72.9%	66.0%	74.6%	72.1%	52.1%	55.9%	63.9%
LPL/**LLP**	42.12	0.46	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
OLO/**OOL**	45.94	0.44	94.6%	99.9%	98.6%	95.7%	87.5%	97.8%	94.7%	70.9%	75.5%	85.0%
PLP/PPL	47.47	0.51	2.0%	4.1%	1.5%	1.1%	1.1%	0.3%	1.3%	2.1%	2.5%	1.3%
OPO/**OOP**	49.35	0.30	-	100.0%	-	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
POP/PPO	50.45	0.49	-	-	-	4.7%	2.9%	2.6%	2.6%	3.3%	1.5%	0.6%
OSO/**OOS**	52.59	1.03	-	-	-	-	-	-	-	-	96.9%	78.8%

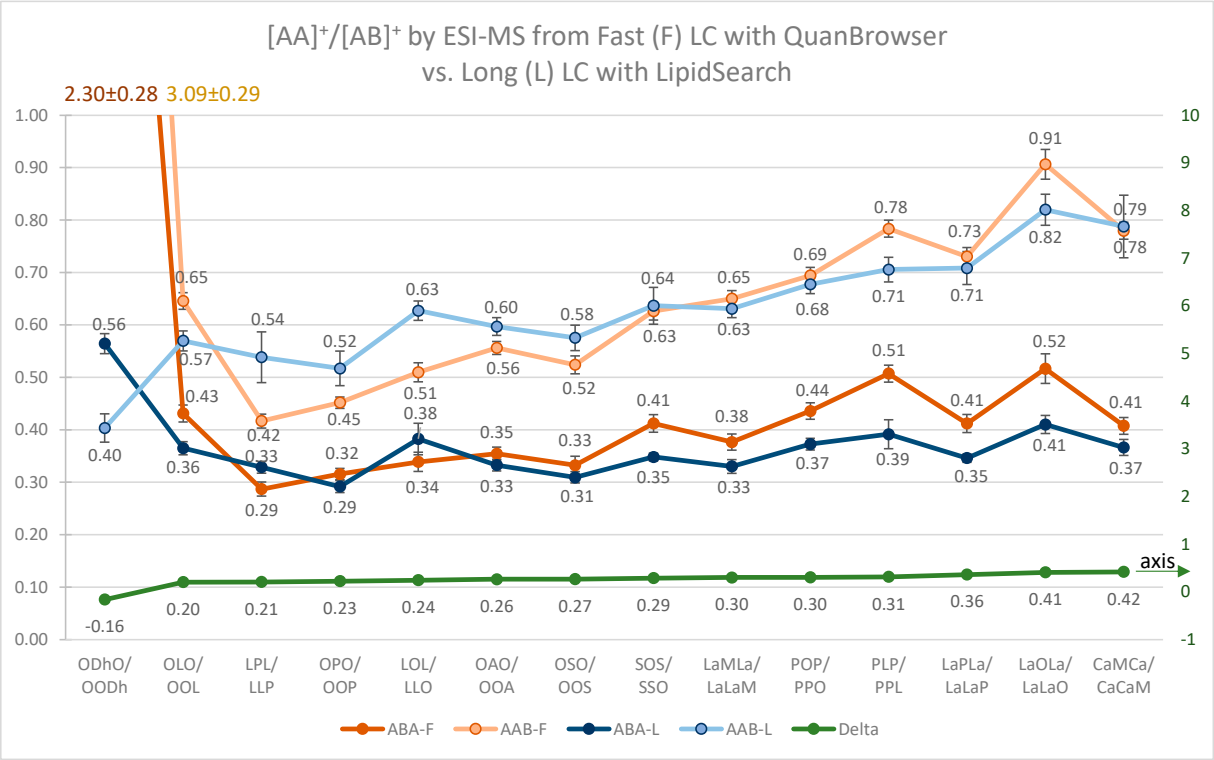
[2]D(2): ESI-MS (QB)			Pinto Beans	Navy Beans	Cranberry Beans	Black Beans	Black-Eyed Peas	Baby Lima Beans	Butter Beans	Lentils	Green Split Peas	Garbanzo Beans
TAG	RT	SD	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB	%AAB
LOL/**LLO**	41.68	± 0.39	91.2%	86.5%	84.9%	83.0%	71.7%	84.9%	83.5%	58.8%	66.3%	80.6%
LPL/**LLP**	42.12	± 0.46	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
OLO/**OOL**[b]	46.12	± 0.26	67.7%	**100.0%**	**97.8%**	53.8%	-	35.0%	-	**93.3%**	**100.0%**	80.6%
PLP/PPL	47.64	± 0.32	5.5%	11.6%	4.1%	5.4%	4.6%	1.8%	4.6%	5.8%	6.9%	5.1%
OPO/**OOP**	49.31	± 0.33	-	100.0%	-	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
POP/PPO	50.42	± 0.49	-	-	-	6.9%	5.1%	4.8%	4.8%	5.5%	3.7%	2.9%
OSO/**OOS**	53.28	± 0.26	-	-	-	-	-	-	-	88.5%	100.0%	94.5%

[a]$[OS]^+$ fragment smaller than normal; $[OO]^+/[OS]^+$ out of range.

[b]One of two CR2 values was high for bolded values.



Supplementary Figure S1. Structures of molecules having calculated *m*/*z* values of 324.2897 and 324.3261.



Supplementary Figure S2. Critical Ratio 2 (CR2), $[AA]^+/[AB]^+$, of pulse triacylglycerols by electrospray ionization (ESI) high-resolution, accurate-mass (HRAM) mass spectrometry (MS) sorted by the difference between CR2 for ABA versus AAB TAG regioisomers, ΔCR2. The ΔCR2 values (in green, right axis) from only the current data are listed. CR2 values are compared to those from fast chromatography, previously reported [3]. CR2 for ODhO/OODh not shown for fast LC APPI-MS data. Fatty acid abbreviations in Materials and Methods.