Supplementary Information for:

Multi-Matrix, Dual Polarity, Multiplex Mass Spectrometry Imaging Strategy Applied to a Germinated Maize Seed: Toward Mass Spectrometry Imaging of an Untargeted Metabolome

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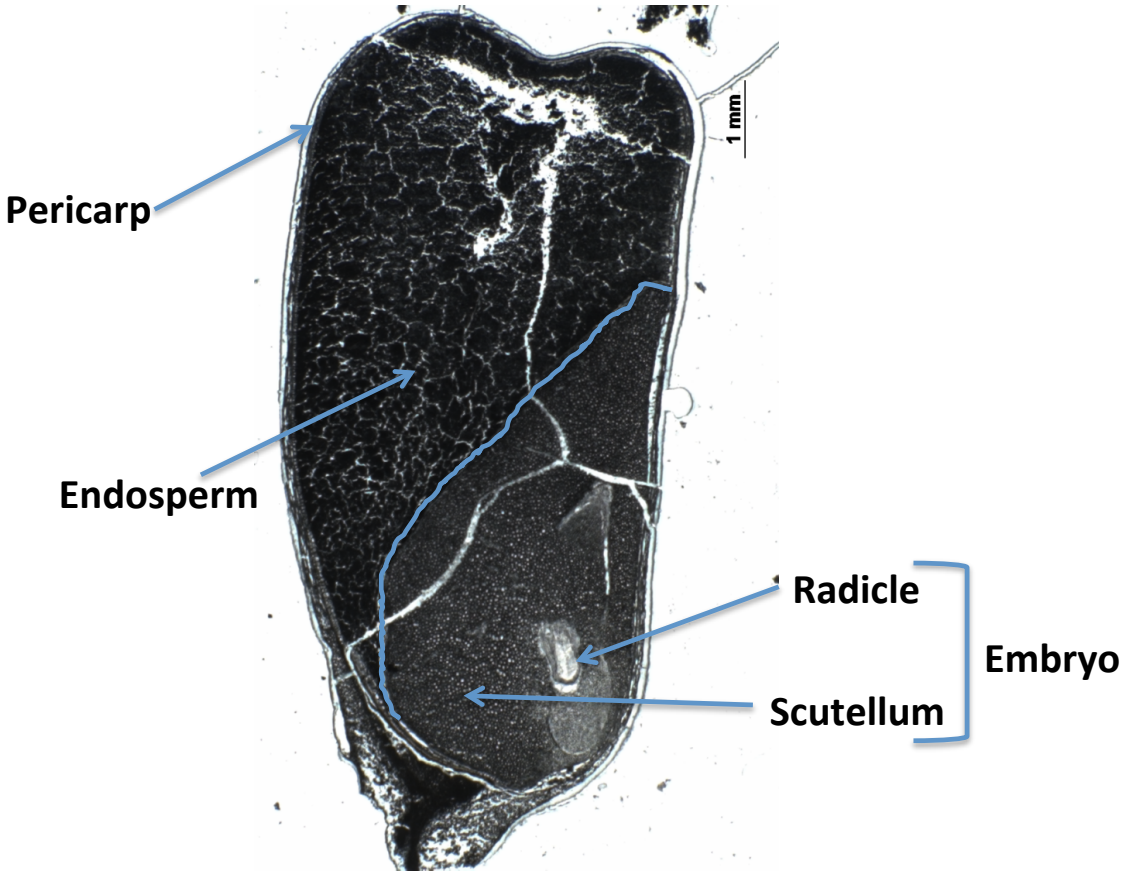
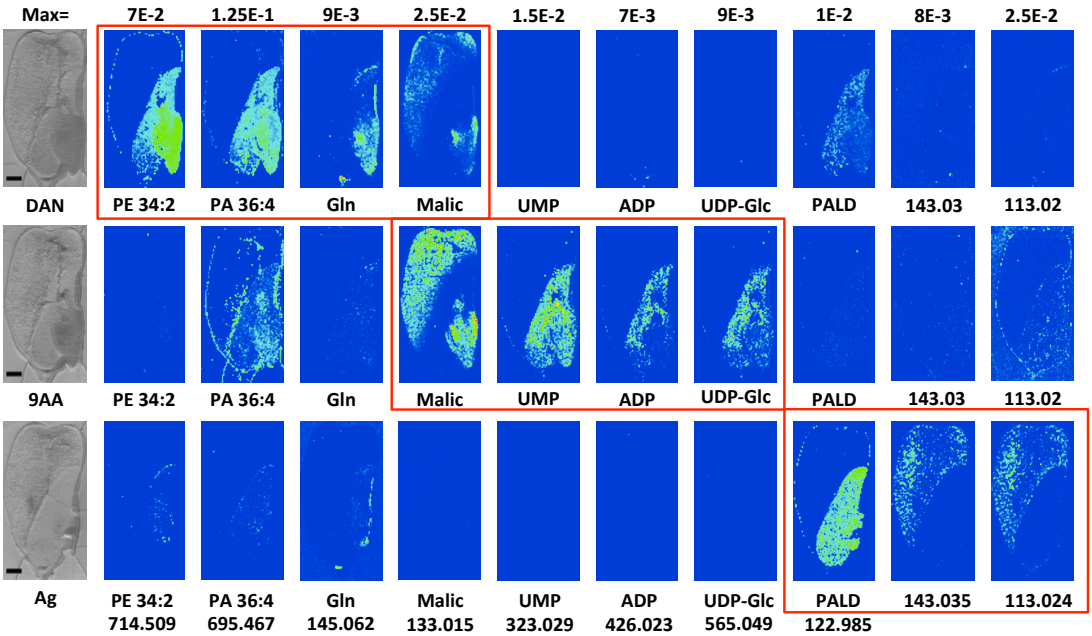
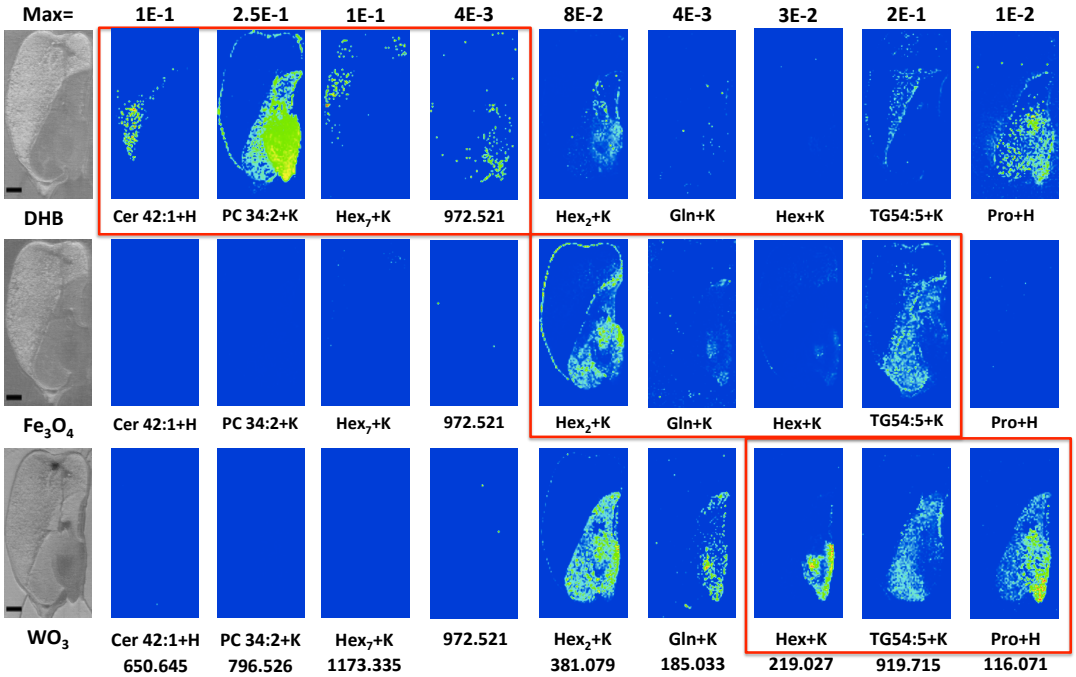


Supplementary Figure 1: Anatomy of maize seed. Pericarp: Outer layers of the seed that protect the seed. Endosperm: Starchy storage area. Scutellum: Oil storage area and metabolically active region during germination. Radicle: Primary root. Coleoptile, protective sheath of emerging shoot and also a part of embryo, is not seen yet in this image. The blue line through the seed serves as a visual representation of the boundary between the embryo and endosperm.

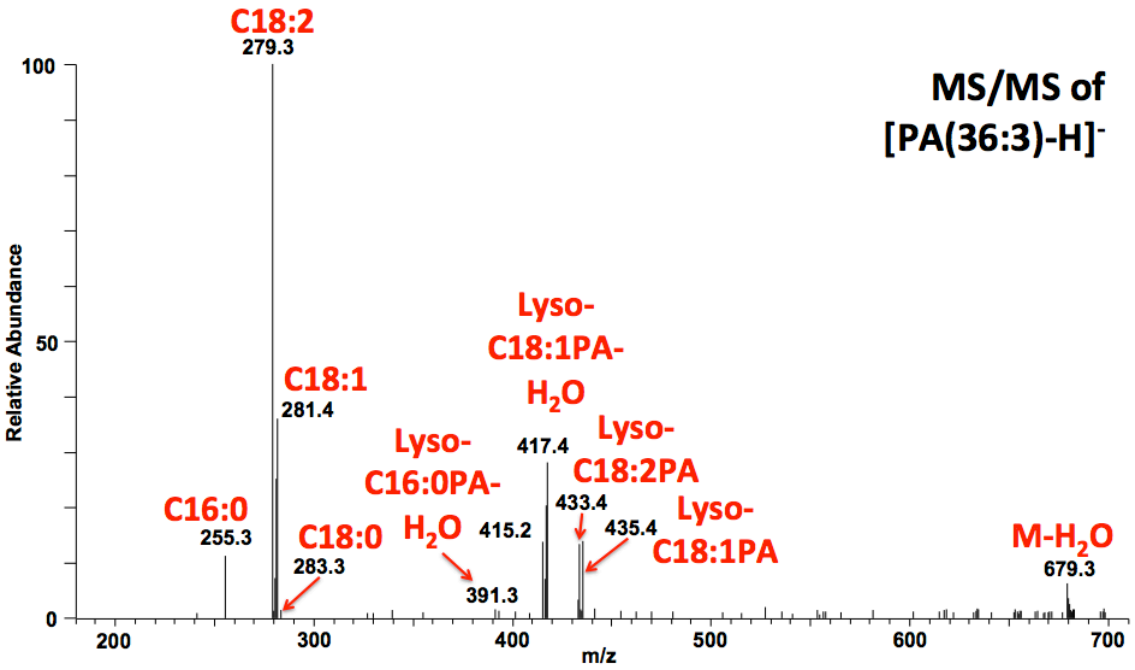
A)



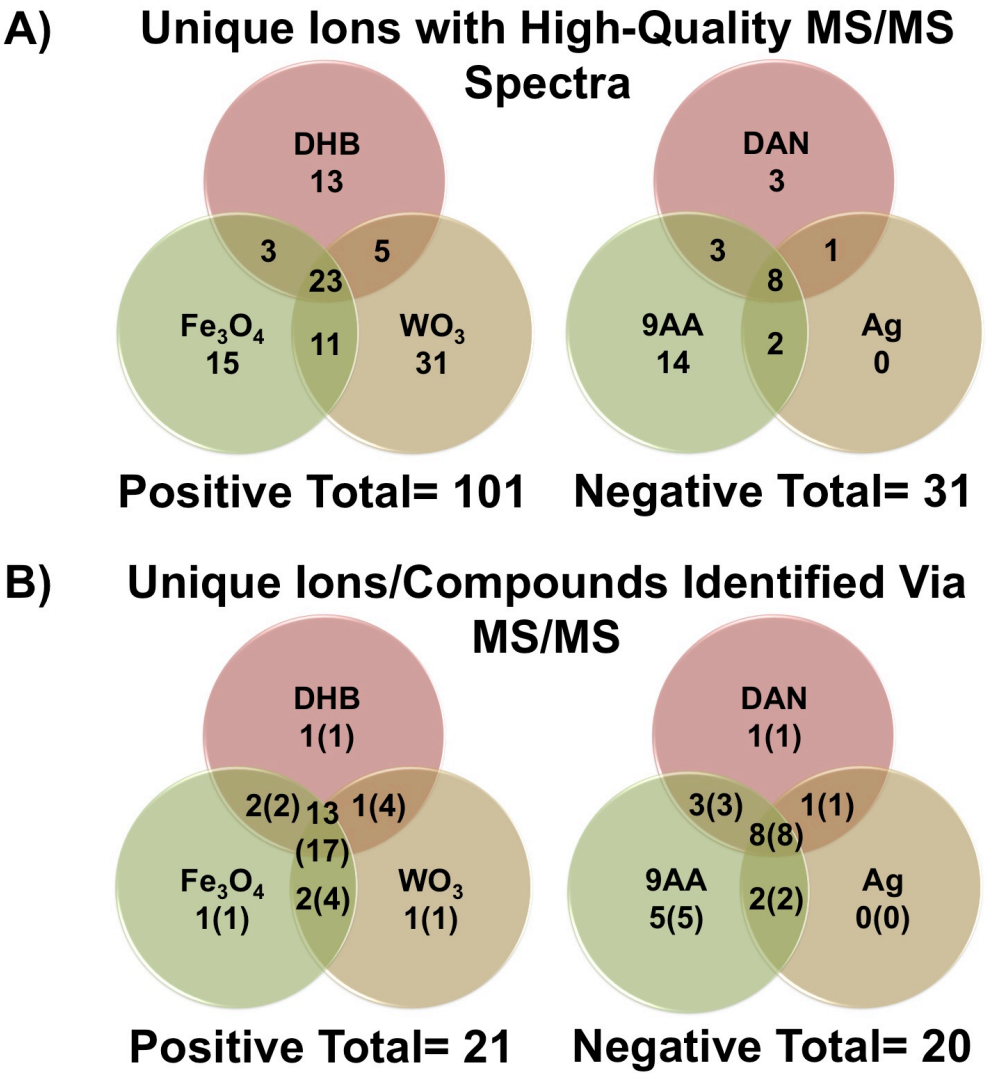
B)



Supplementary Figure 2. Comparison of matrix effectiveness for various classes of compounds. The same images shown in Figure 2 for negative mode (A) and Figure 3 for positive mode (B) but normalized against total ion count with maximum values adjusted for each compound. Scale bars represent 1mm.



Supplementary Figure 3. An average of 91 MS/MS spectra for PA (36:3) at *m/z* 697.483 using DAN as a matrix in negative ion mode. Fragments include C16:0 *(m/z* 255.3), C18:2 (*m/z* 279.3), C18:1 (*m/z* 281.4), and C18:0 (*m/z* 283.3) fatty acid chains, along with several corresponding Lyso-PA fragments. This MS/MS spectrum is considered as a mixture of PA (16:0/20:3), PA (18:1/18:2), and PA (18:0/18:3).



Supplementary Figure 4. The same as Figure 4 but in a replicate experiment with another seed, to demonstrate the robustness of the method. A lower number of small molecules were detected in negative mode. This was attributed to biological variations between the seeds.

Supplementary Table 1: All 104 unique positive ions with high quality MS/MS spectra that passed through MS/MS filtering. Highlighted are ions identified via MS/MS, as assigned in Suppl. Table 3.

All Three	DHB/Fe_3O_4	WO_3/Fe_3O_4	WO_3/DHB	DHB Only	Fe_3O_4 Only		WO_3 Only
184.073	212.852	749.393	739.469	198.089	112.896	706.612	600.763
381.080	765.485	785.472	901.218	222.029	119.930	713.455	631.142
601.520	777.427	903.740		230.946	120.926	727.731	647.092
603.535	792.435	917.701		268.901	154.027	745.238	649.095
735.437	824.559	921.731		614.910	156.886	751.411	651.098
737.453	911.446			637.458	185.042	773.394	655.470
775.410				645.166	274.808	787.468	687.052
796.526				650.644	310.913	798.545	741.154
820.526				692.619	379.203	816.436	761.453
822.542				697.343	607.011	831.510	808.571
893.701				756.554	611.179	833.253	935.712
895.717				758.570	626.529	833.494	949.692
919.716				763.469	629.143	877.726	
				784.585	631.411	879.741	
				786.600	635.413	881.755	
				913.447	638.550	901.365	
				913.704	645.116	901.727	
				1011.281	654.511	905.757	
				1173.337	659.502	907.771	
				1497.440	671.200	917.338	
					681.220	995.306	
					699.195	1169.735	
					699.699	1331.145	

Supplementary Table 2: All 62 unique negative ions with high quality MS/MS spectra that passed through MS/MS filtering. Highlighted are ions identified via MS/MS, as assigned in Supp. Table 4.

All Three	DAN/9AA	DAN/Ag	Ag/9AA	DAN Only	9AA Only	Ag Only
279.233	133.015	255.233		146.046	323.029	181.050
714.507	191.020	671.465		306.076	402.995	629.549
857.520	689.215	697.481		674.328	606.075	673.481
859.535	758.178	738.505		675.443	611.145	713.054
	869.280	742.538		695.467	658.855	745.500
	1031.333	768.553		699.499	793.516	817.512
	1423.815	833.518		707.228	809.520	835.530
	1439.812	861.245		709.366	851.269	846.709
		861.549		721.503	924.657	
				736.495	984.604	
				740.524	1038.689	
				831.506	1070.715	
				865.464	1193.388	
				1013.328	1200.743	
				1029.768	1355.440	
					1407.819	
					1465.825	
					1517.495	

Supplementary Table 3. Tentatively identified compounds in positive mode summarized in Figure 4B.

m/z[a]	Suspected Identity[b]	Species Observed[a]	Mass Error[c] (ppm)	*m/z* of fragment ions and assignments[d]
184.074 222.029	Phosphocholine†‡	+H +K	0.9, 0.8, 1.5 -0.1, XX, XX	125 (-$N(CH_3)_3$), 86(-Phos) 163 (-$N(CH_3)_3$)
198.089	Methacholine†	+K	-0.7, XX, XX	139 (-$N(CH_3)_3$)
381.080	Disaccharide†	+K	-0.3, 2.6, 0.9	219 (-Hex-H_2O), 201 (-Hex)
601.519	DAG 36:3 Frag	+H	0.3, 0.4, 1.0	319 (-18:1), 345 (-16:0)
603.535	DAG 36:2 Frag	+H	0.6, 0.4, 1.5	347 (-16:0)
659.502	DAG 36:2	+K	XX, 0.6, XX	403 (-16:0), 379 (-18:2)
650.644	Cer 42:1†	+H	-1.6, XX, XX	300 (-24:0), 282 (300-H_2O), 264 (282-H_2O), 368 (-18:1dOH) See Figure 6 for structures
671.200	Hex 4	-H_2O+Na	XX, 0.7, XX	509.20 (-Hex-H_2O), 347.18 (-2Hex-2H_2O)
735.437 751.411	PC 32:0	-$N(CH_3)_3$+Na+K -$N(CH_3)_3$+2K	3.5, 5.0, 4.1 XX, 4.1, XX	611 (-(PCho-$N(CH_3)_3$)), 479 (-16:0) 495 (-16:0)
737.453 796.526 758.570	PC 34:2	-$N(CH_3)_3$+K+H +K +H	0.3, 3.0, 1.2 0.7, 2.1, 0.9 0.3, XX, XX	613 (-(PCho-$N(CH_3)_3$)), 481 (-16:0) 737 (-$N(CH_3)_3$), 613 (-PCho), 540 (-16:0), 516 (-18:2) 699 (-$N(CH_3)_3$), 575 (-PCho), 502 (-16:0), 478 (-18:2)
739.470 777.427 798.545 761.453	PC 34:1	-$N(CH_3)_3$+K+H -$N(CH_3)_3$+2K +K -$N(CH_3)_3$+Na+K	3.9, XX, 2.5 4.2, 4.8, XX XX, 4.5, XX XX, XX, 4.0	615 (-(PCho-$N(CH_3)_3$)), 483 (-16:0) 734 (-C_2H_4O), 521 (-16:0), 495 (-18:1), 493 (-18:0) 739 (-$N(CH_3)_3$) 637 (-(PCho-$N(CH_3)_3$)), 505 (-16:0)
763.469	PC 34:0	-$N(CH_3)_3$+Na+K	5.3, XX, XX	639 (-(PCho-$N(CH_3)_3$))
820.526	PC 36:4	+K	0.6, 1.9, 0.9	761 (-$N(CH_3)_3$), 637 (-PCho), 564 (-16:0), 540 (-18:2), 538 (-18:1)
822.542 784.585	PC 36:3	+K +H	-2.0, 1.9, 0.8 -0.3, XX, XX	763 (-$N(CH_3)_3$), 639 (-PCho), 566 (-16:0), 542 (-18:2), 540 (-18:1) 725 (-$N(CH_3)_3$), 504 (-18:2), 502 (-18:1)
824.559 786.600	PC 36:2	+K +H	2.0, 4.7, XX -1.0, XX, XX	765 (-$N(CH_3)_3$), 641 (-PCho) 506 (-18:2), 504 (-18:1)

893.701 877.726	TAG 52:4	+K +Na	1.0, 2.2, 1.8 XX, 1.0, XX	637 (-16:0), 613 (-18:2), 319 (-DAG 34:2) 621 (-16:0), 597 (-18:2), 575 (DAG 34:2 Frag+H)
895.716 879.741	TAG 52:3†	+K +Na	0.4, 2.3, 1.9 XX, -0.1, XX	639 (-16:0), 615 (-18:2), 613 (-18:1) 623 (-16:0), 599 (-18:2), 597 (-18:1), 577 (DAG 34:1 Frag+H), 575 (DAG 34:2 Frag+H)
881.755	TAG 52:2	+Na	XX, -1.6, XX	625 (-16:0), 599 (-18:1), 577 (DAG 34:1 Frag+H)
917.701 901.727	TAG 54:6‡	+K +Na	XX, 1.8, 1.5 XX, 0.8, XX	661 (-16:0), 637 (-18:2), 655 (-(18:2-H2O)) 621 (-18:2), 599 (DAG 36:4 Frag+H)
919.716	TAG 54:5‡	+K	1.0, 1.0, 1.6	663 (-16:0), 639 (-18:2), 637 (-18:1)
921.732 905.757	TAG 54:4‡	+K +Na	XX, 0.7, 1.7 XX, -0.6, XX	641 (-18:2), 639 (-18:1) 625 (-18:2), 623 (-18:1), 601 (DAG 36:3 Frag+H)
907.771	TAG 54:3	+Na	XX, -1.8, XX	651 (-16:0), 625 (-18:1), 623 (-18:0), 333 (-DAG 34:2)
903.740	TAG 56:8	+H	XX, -2.4, -3.7	623 (-18:2), 621 (-18:1), 601 (DAG 36:3 Frag+H)
833.253	Hexose 5	$-H_2O+Na$	XX, 2.0, XX	671 (-(Hex-H_2O)), 509 (-(2Hex-2H_2O)), 347 (-(3Hex-3H_2O))
1011.281 995.306	Hexose 6†	$-H_2O+K$ $-H_2O+Na$	0.7, XX, XX XX, 0.3, XX	849 (-(Hex-H_2O)), 687 (-(2Hex-2H_2O)) 833 (-(Hex-H_2O)), 671 (-(2Hex-2H_2O)), 509 (-(3Hex-3H_2O))
1173.337	Hexose 7†	$-H_2O+K$	0.0, XX, XX	1011 (-(Hex-H_2O)), 849 (-(2Hex-2H_2O))
1497.440	Hexose 9	$-H_2O+K$	1.0, XX, XX	1335 (-(Hex-H_2O)), 1173 (-(2Hex-2H_2O)), 1011 (-(3Hex-3H_2O))

a. The same compound detected as various adducts were grouped together and species observed were shown.
b. Assignments were based on accurate mass search on Metlin and manual MS/MS interpretation. † and ‡ denote when MS/MS spectra were available in Metlin and MassBank database, respectively, and used for comparison.
c. Mass errors are shown in the order of DHB, Fe_3O_4 NP and WO_3 NP matrix. XX represents that there is no peak for the given matrix.
d. Fragment assignments are shown in the parenthesis. $N(CH_3)_3$: choline head group. Phos: phosphoric acid (H_3PO_4). Hex: hexose (e.g., glucose). (Hex-H_2O): water loss from hexose. PCho: phosphocholine head group. PCho-$N(CH_3)_3$: Phosphocholine head group with trimethyl amine loss. 16:0, 18:0, 18:1, 18:2: C16:0, C18:0, C18:1 and C18:2 fatty acid, respectively.

Supplementary Table 4. Tentatively identified compounds in negative mode summarized in Figure 4B.

m/z	Suspected Identity[a]	Species Observed	Mass Error[b] (ppm)	m/z of fragment ions and assignments[c]
133.015	Malic Acid†‡	-H	4.5, 3.7, XX	87, 71
146.046	Glutamic Acid†‡	-H	3.1, XX, XX	128 (-H_2O), 102 (-CO_2)
191.020	Citric Acid/Isocitrate†‡	-H	1.1, 1.7, XX	111, 87
306.076	Glutathione†‡	-H	-0.2, XX, XX	288, 272, 254
323.029	Uridine monophosphate†‡	-H	XX, -0.3, XX	280, 211
402.995	Uridine diphosphate†‡	-H	XX, 0.5, XX	385, 305, 273
606.075	UDP-N-acetylglucosamine†‡	-H	XX, 1.6, XX	403, 385, 282, 273
658.855	Phytic Acid	-H	XX, 0.8, XX	561 (-Phos), 463 (-2 Phos)
671.464	PA 34:2	-H	0.0, XX, -3.2	415 (-16:0), 409 (-(18:2-H_2O)), 391 (-18:2), 279 (18:2-H), 255 (16:0-H)
695.467	PA 36:4	-H	2.1, XX, XX	433 (-(18:2-H_2O)), 415 (-18:2), 279 (18:2-H)
697.480	PA 36:3	-H	2.9, XX, -2.8	417 (-18:2), 415 (-18:1), 281 (18:1-H), 279 (18:2-H)
714.508	PE 34:2	-H	2.3, -0.0, -2.6	452 (-(18:2-H_2O)), 434 (-18:2), 279 (18:2-H), 255 (16:0-H)
738.505	PE 36:4‡	-H	2.5, XX, -4.4	476 (-(18:2-H_2O)), 279 (18:2-H)
742.540	PE 36:2‡	-H	2.5, XX, -4.2	480 (-(18:2-H_2O)), 462 (-18:2), 281 (18:1-H), 279 (18:2-H), 255 (16:0-H)
768.553	PE 38:3	-H	1.7, XX, -2.8	281 (18:1-H), 279 (18:2-H)
721.503	PG 32:0	-H	1.3, XX, XX	483 (-(16:0-H_2O)), 465 (-16:0), 391 (LPA16:0-H_2O), 255 (-16:0)
745.500	PG 34:2	-H	XX, XX, -3.2	279 (18:2-H), 255 (16:0-H)
831.506	PI 34:3‡	-H	3.4, XX, XX	553 (-18:3), 391 (LPA 16:0-H_2O), 277 (18:3-H), 255 (16:0-H), 241 (Inositol 6-phosphate-H_2O)

833.517	PI 34:2‡	-H	3.2, XX, -2.4	577 (-16:0), 571 (-(18:2-H_2O)), 553 (-18:2), 391 (LPA 16:0-H_2O), 279 (18:2-H), 255 (16:0-H), 241 (Inositol 6-Phosphate-H_2O)
835.530	PI 34:1‡	-H	XX, XX, -4.9	579 (-16:0), 571 (-(18:1-H_2O)), 553 (-18:1), 391 (LPA16:0-H_2O), 281 (18:1-H), 255 (16:0-H)
857.520	PI 36:4	-H	2.7, 1.9, -1.3	595 (-(18:2-H_2O)), 577 (-18:2), 415 (LPA18:2-H_2O), 279 (18:2-H), 241 (Inositol 6-phosphate-H_2O)
859.535	PI 36:3‡	-H	1.7, 2.1, -2.6	579 (-18:2), 577(-18:1) 417 (LPA18:1-H_2O), 281 (18:1-H), 279 (18:2-H)
861.550	PI 36:2‡	-H	2.4, XX, -3.0	581 (-18:2), 579 (-18:1), 283 (18:0-H)
793.516	SQDG 32:0	-H	XX, 1.8, XX	537 (-16:0)
817.512	SQDG 34:2	-H	XX, XX, -2.5	561 (-16:0), 537 (-18:2)

a. Assignments were based on accurate mass search on Metlin and manual MS/MS interpretation. † and ‡ denote when MS/MS spectra were available in Metlin and MassBank database, respectively, and used for comparison.
b. Mass errors are shown in the order of DAN, 9AA, and Ag NP matrix. XX represents there is no peak for the given matrix.
c. Fragment assignments are shown in the parenthesis. Phos: phosphoric acid (H_3PO_4). LPA: lysophosphatidic acid species with fatty acid chain denoted. 16:0, 18:0, 18:1, 18:2, 18:3: C16:0, C18:0, C18:1, C18:2, and C18:3 fatty acid, respectively. Masses without easily assigned fragments are not labeled. Rather, matching structures can be found online at the Metlin or MassBank databases.