Electronic Supplementary Information for :

Direct Analysis of Metabolites in the Liver Tissue of Zebrafish Exposed to Fiproles by Internal Extractive Electrospray Ionization Mass Spectrometry

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Sample preparation:

the collected liver sample from a zebrafish was divided into two parts. The first part (3mg) was mixed with 60 μL of cold methanol/water (4:1, v:v) and then homogenized using a high-speed blender.[1] After 5 minutes of ultrasonication in the ice bath, the sample was placed on ice for 20 minutes and then centrifuge at 12000g for 10 min (4 °C). The collected supernatant was dried by nitrogen blowing and dissolved in 20 μL of methanol/water (4:1, v:v) before d-SPME-iEESI-MS analysis. The second part without any pretreatment was stored at -80 ℃ for next step of direct analysis by iEESI-MS.

Analysis of zebrafish liver tissue by dispersed solid-phase microextraction coupled with iEESI-MS (d-SPME-iEESI-MS):

As reported in previous studies, the Lewis acid site in TiO_2 ($Ti4^+$) has a strong affinity to Lewis basic compounds, such as phospholipids, hydroxyl carboxylic acids, and ceramides.[2] In acidic environment, TiO_2 exhibits the characteristics of Lewis acid, and Lewis basic compounds are adsorbed on TiO_2. While, the Lewis acid-base interactions will be destroyed under alkaline conditions, and basic compounds desorb from the surface of Fe_3O_4@TiO_2 nanocomposite.[3, 4] According to the mechanism of interactions between Lewis basic compounds and TiO_2, d-SPME-iEESI-MS was used to analyze a zebrafish liver sample. The detail process referred to previous literatures[4] as follows: firstly, 0.5 μL of prepared liver extract and 10 μL of Fe_3O_4@TiO_2 nanocomposite water suspension (4 mg/mL, containing 1% TFA) were mixed, and Lewis basic compounds in the mixture adsorbed on Fe_3O_4@TiO_2 nanocomposite. After that, magnetically collected the Fe_3O_4@TiO_2 nanocomposites with the adsorbed Lewis basic compounds by using an external magnetic field. Next, desorb Lewis basic compounds from the Fe_3O_4@TiO_2 nanocomposites by 1 μL of methanol containing 1.5% NH_4OH, and collect the eluted components by the silver-coated ESI tip. Then, the ESI tip was placed horizontally pointing toward the ion inlet at a distance of 5 mm away from the MS ion inlet. Finally, the collected eluted components was charged with +4.5 KV, and the concentrated compounds in zebrafish liver was ionized and detected.

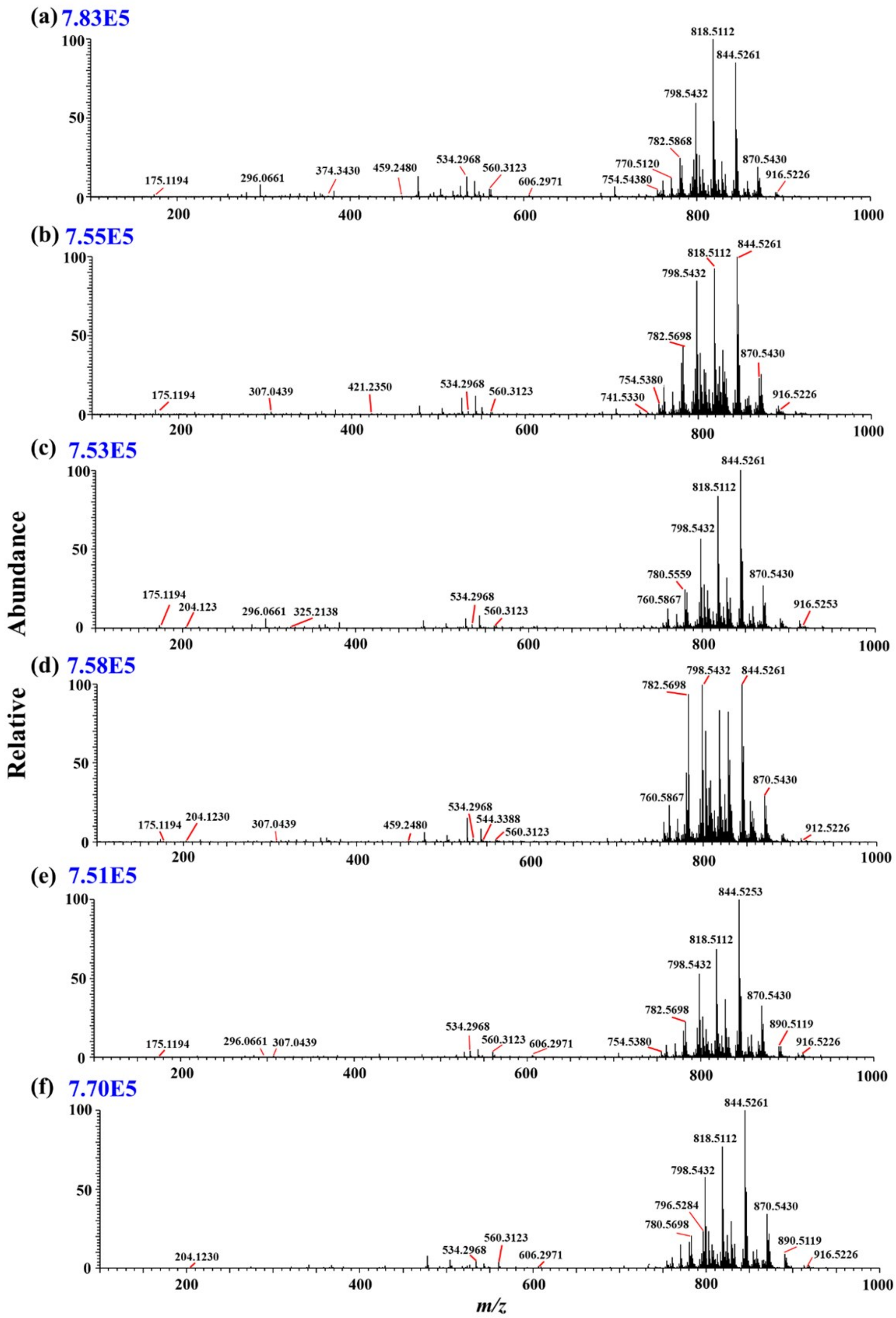

Fig. S1 Mass spectra of liver tissue samples in all tested group detected by iEESI-MS. (a) control group; (b) FP exposed group; (c) FD exposed group; (d) FI exposed group; (e) FO exposed group: (f) MI exposed group.

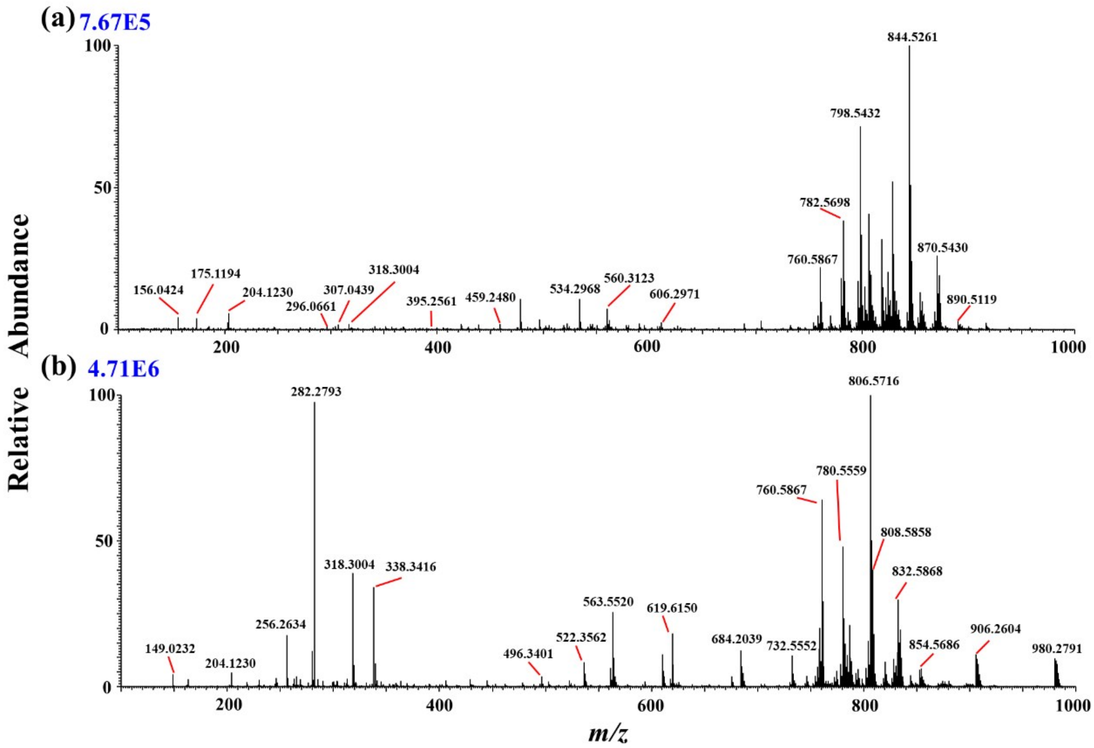

Fig. S2 Mass spectra of liver tissue detected by two methods. (a) detected by iEESI-MS; (b) detected by d-SPME - iEESI-MS.

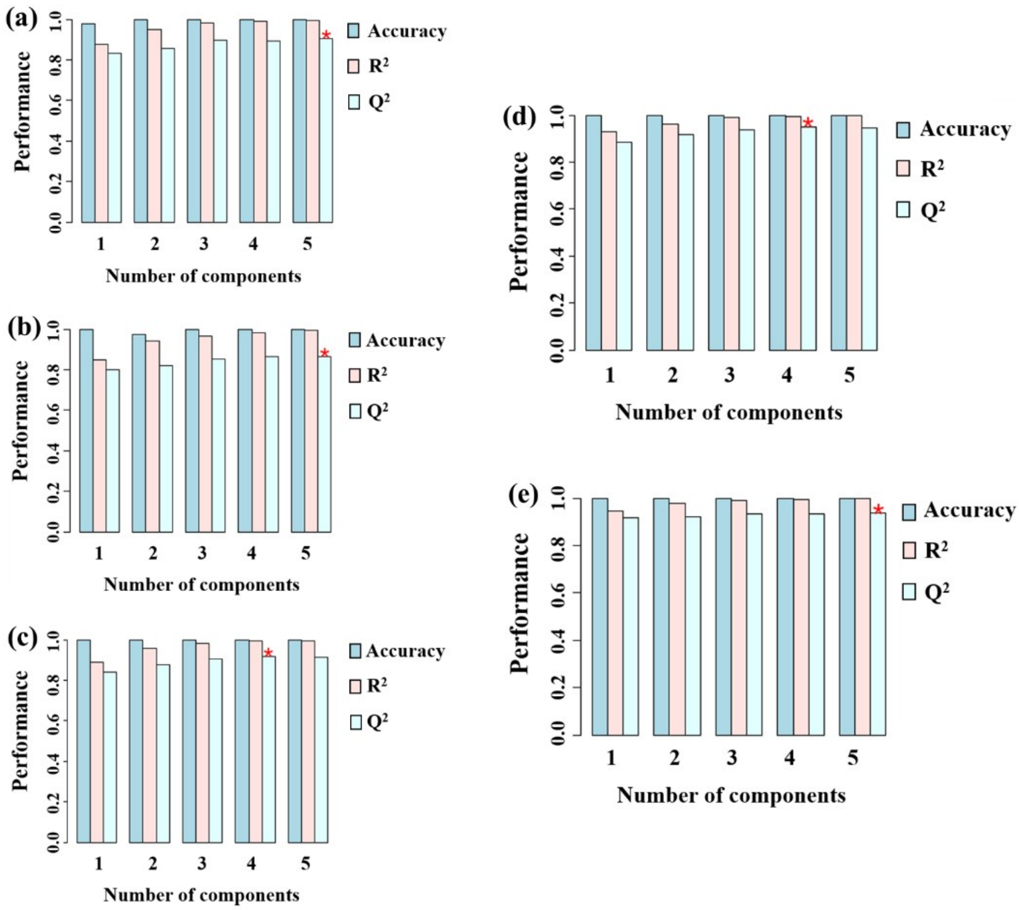

Fig. S3. CV score of PLS-DA model between control group and each exposed group. (a) control group and FP exposed group; (b) control group and FD exposed group; (c) control group and FI exposed group; (d) control group and FO exposed group; (e) control group and MI exposed group.

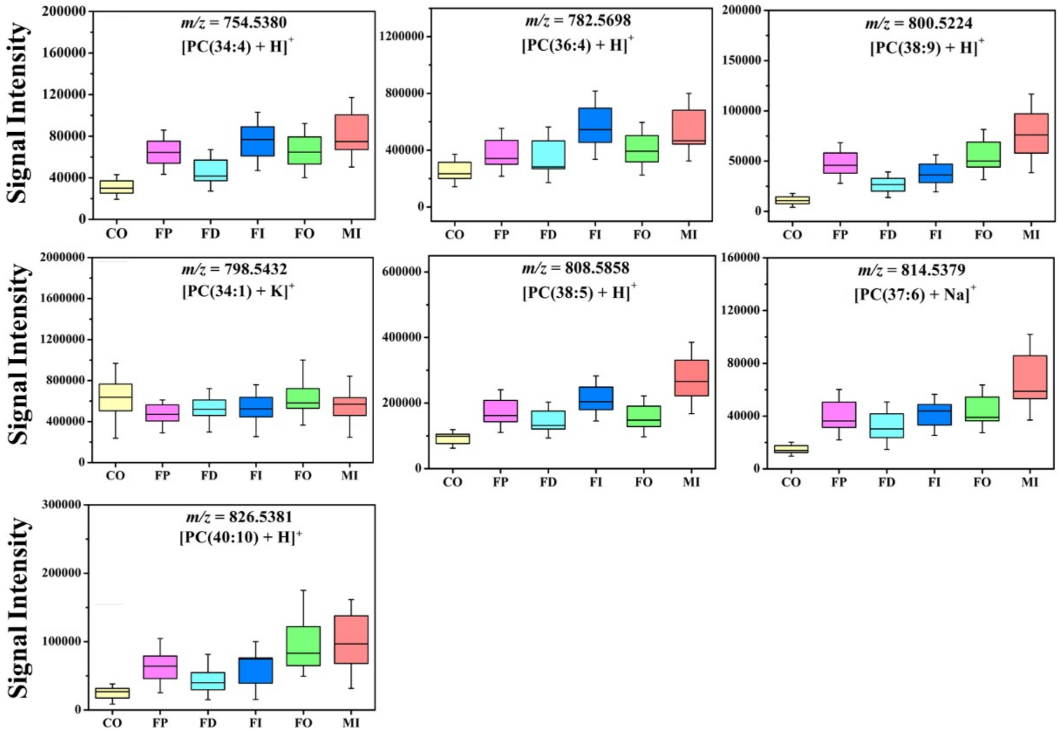

Fig. S4 Box-plot showed the signal intensities of 7 PC signals in the liver tissue samples of control group and exposed groups.

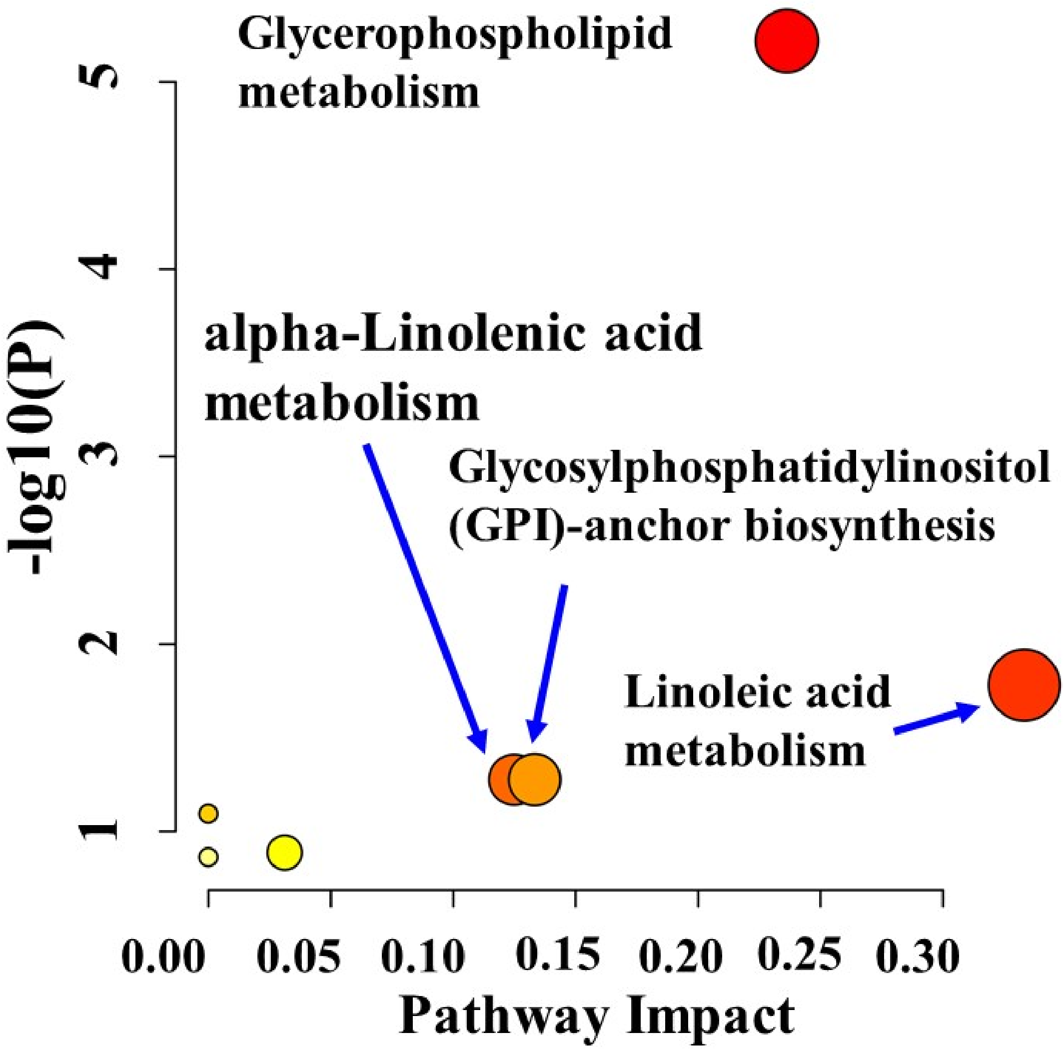

Fig. S5 Pathway analysis of the identified differential signals in FP exposed group.

Table S1. Identification of signals detected by iEESI-MS and d-SPME-iEESI-MS.

No.	Experimental *m/z*	Theoretical *m/z*	Error (ppm)	Compound name	Formula	Ion Formation	MS/MS Fragments	Detected method
1	149.0232	149.0239	5	Phthalic acid	$C_8H_6O_4$	$[M+H-H_2O]^+$	93.0/121.0	d-SPME - iEESI-MS
2	175.1194	175.1190	3	L-Arginine/D-Arginine	$C_6H_{14}N_4O_2$	$[M+H]^+$	60.1/70.1/116.1	iEESI-MS
3	204.1230	204.1230	0	N-Lactoylleucine	$C_9H_{17}NO_4$	$[M+H]^+$	186.1/158.1/57.1	both
4	282.2793	282.2790	1	Oleamide	$C_{18}H_{35}NO$	$[M+NH_4-H_2O]^+$	321.3/303.3	d-SPME - iEESI-MS.
5	296.0661	296.0660	0	Glycerophosphocholine	$C_8H_{20}NO_6P$	$[M+K]^+$	86.1/104.1	iEESI-MS
6	307.0439	307.0448	3	2-(3,4 Dihydroxybenzoyloxy)-4,6-dihydroxybenzoate	$C_{14}H_{10}O_8$	$[M+H]^+$	289.0/245.0/275.0	both
7	318.3004	318.3003	0	Phytosphingosine	$C_{18}H_{39}NO_3$	$[M+H]^+$	300.3/60.0	both
8	325.2138	325.2138	0	Eicosapentaenoic acid	$C_{20}H_{30}O_2$	$[M+Na]^+$	189.2/307.2	iEESI-MS
9	338.3416	338.3412	1	Erucic acid	$C_{22}H_{42}O_2$	$[M+NH_4-H_2O]^+$	321.3/303.3	d-SPME - iEESI-MS
10	374.3430	374.3423	1	Dihomo-γ-linolenoylcholine	$C_{25}H_{46}NO_2$	$[M+H-H_2O]^+$	88.1/356.3	iEESI-MS
11	379.2041	379.2034	2	Plastoquinone 3	$C_{23}H_{32}O_2$	$[M+K]^+$	323.2/259.2/281.2	iEESI-MS

12	395.2561	395.2557	1	LysoPA(P-16:0/0:0)	$C_{19}H_{39}O_6P$	$[M+H]^+$	377.2/297.3	iEESI-MS
13	400.378	400.3785	1	Oleoylcholine	$C_{23}H_{46}NO_2$	$[M+CH_3OH+H]^+$	88.1/382.4	both
14	415.2110	415.2115	1	Eplerenone	$C_{24}H_{30}O_6$	$[M+H]^+$	345.2/327.2	d-SPME - iEESI-MS
15	421.235	421.2351	0	Misoprostol	$C_{22}H_{38}O_5$	$[M+K]^+$	389.4/403.4/59.1	iEESI-MS
16	459.2480	459.2482	0	DHAP (18:0)	$C_{21}H_{41}O_7P$	$[M+Na]^+$	81.0/57.0/441.3	both
17	496.3401	496.3398	1	LysoPC (16:0)	$C_{24}H_{50}NO_7P$	$[M+H]^+$	184.1/86.1/104.1	both
18	522.3562	522.3554	2	LysoPC (18:1)	$C_{26}H_{52}NO_7P$	$[M+H]^+$	184.1/86.1/104.1	both
19	532.2816	532.2800	3	LysoPC (16:1)	$C_{24}H_{48}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	both
20	534.2968	534.2956	3	LysoPC (16:0)	$C_{24}H_{50}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	iEESI-MS
21	544.3388	544.3398	1	LysoPC (20:4)	$C_{28}H_{50}NO_7P$	$[M+H]^+$	184.1/86.1/104.1	both
22	548.3124	548.3113	2	LysoPC (17:0)	$C_{25}H_{52}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	iEESI-MS
23	556.2815	556.2800	2	LysoPC (18:3)	$C_{26}H_{48}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	iEESI-MS
24	558.2971	558.2956	2	LysoPC (18:2)	$C_{26}H_{50}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	iEESI-MS
25	560.3123	560.3113	2	LysoPC (18:1)	$C_{26}H_{52}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	both

26	562.3280	562.3269	2	LysoPC (18:0)	$C_{26}H_{54}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	both
27	563.5520	563.5504	3	Cer (d18:1/18:1)	C36H69NO3	$[M+NH_4-H_2O]^+$	282.2797	d-SPME - iEESI-MS
28	580.2815	580.2800	2	LysoPC (20:5)	$C_{28}H_{48}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	iEESI-MS
29	606.2971	606.2956	2	LysoPC (22:6)	$C_{30}H_{50}NO_7P$	$[M+K]^+$	184.1/86.1/104.1	iEESI-MS
30	619.6150	619.6182	5	Glycinoprenol 9	$C_{45}H_{80}O$	$[M+H-H_2O]^+$	57.0/67.0/71.1	d-SPME - iEESI-MS
31	653.4560	653.4542	3	DG (36:5)	$C_{39}H_{66}O_5$	$[M+K]^+$	529.4/609.4	iEESI-MS
32	741.5330	741.5307	3	SM (d18:0/16:1)	$C_{39}H_{79}N_2O_6P$	$[M+K]^+$	184.1/86.1/104.1	both
33	754.5380	754.5381	0	PC (34:4)	$C_{42}H_{76}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
34	758.5711	758.5694	2	PC (34:2)	$C_{42}H_{80}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
35	760.5867	760.5851	2	PC (34:1)	$C_{42}H_{82}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
36	766.5390	766.5381	1	PC (35:5)	$C_{43}H_{76}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
37	770.5120	770.5097	3	PC (32:1)	$C_{40}H_{78}NO_8P$	[M+K]+	184.1/86.1/104.1	both
38	774.5062	774.5068	1	PC (36:8)	$C_{44}H_{72}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
39	778.5387	778.5381	1	PC (36:6)	$C_{44}H_{76}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both

40	780.5559	780.5538	3	PC (36:5)	$C_{44}H_{78}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
41	782.5698	782.5694	0	PC (36:4)	$C_{44}H_{80}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
42	788.5225	788.5225	0	PE (40:8)	$C_{45}H_{74}NO_8P$	$[M+H]^+$	647.5	both
43	794.5123	794.5097	3	PC (34:3)	$C_{42}H_{78}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
44	796.5284	796.5282	0	PE (42:9)	$C_{47}H_{76}NO_8P$	$[M+H-H_2O]^+$	673.5	both
45	796.5848	796.5851	0	PC (37:4)	$C_{45}H_{82}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
46	798.5432	798.5410	3	PC (34:1)	$C_{42}H_{82}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
47	800.5224	800.5225	0	PC (38:9)	$C_{46}H_{74}NO_8P$	$[M+H]^+$	86.1/184.1//741.4	both
48	802.5382	802.5381	0	PC (38:8)	$C_{46}H_{76}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
49	806.5720	806.5694	3	PC (38:6)	$C_{46}H_{80}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
50	808.5858	808.5851	1	PC (38:5)	$C_{46}H_{82}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
51	812.559	812.5566	3	PC (35:1)	$C_{43}H_{84}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
52	814.5379	814.5357	3	PC (37:6)	$C_{45}H_{78}NO_8P$	$[M+Na]^+$	184.1/86.1/104.1	both
53	816.5535	816.5538	1	PC (39:8)	$C_{47}H_{78}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both

54	818.5112	818.5097	2	PC (36:5)	$C_{44}H_{78}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
55	826.5381	826.5381	0	PC (40:10)	$C_{48}H_{76}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
56	828.5536	828.5538	0	PC (40:9)	$C_{48}H_{78}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
57	830.5686	830.5694	1	PC (40:8)	$C_{48}H_{80}NO_8P$	[M+H]+	184.1/86.1/104.1	both
58	832.5868	832.5851	2	PC (40:7)	$C_{48}H_{82}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
59	834.6015	834.6007	1	PC (40:6)	$C_{48}H_{84}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
60	842.5120	842.5097	3	PC (38:7)	$C_{46}H_{78}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
61	844.5261	844.5253	1	PC (38:6)	$C_{46}H_{80}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
62	848.5535	848.5566	4	PC (38:4)	$C_{46}H_{84}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
63	854.5686	854.5694	1	PC (42:10)	$C_{50}H_{80}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
64	856.5844	856.5851	1	PC (42:9)	$C_{50}H_{82}NO_8P$	$[M+H]^+$	184.1/86.1/104.1	both
65	858.5430	858.541	2	PC (39:6)	$C_{47}H_{82}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
66	870.5430	870.541	2	PC (40:7)	$C_{48}H_{82}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both
67	890.5119	890.5097	3	PC (42:11)	$C_{50}H_{78}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both

68	916.5226	916.5253	3	PC (44:12)	$C_{52}H_{80}NO_8P$	$[M+K]^+$	184.1/86.1/104.1	both

Table S2. Differential signals in each exposed group.

Group	Differential signals
FP exposed group	LysoPC(16:0), Glycerophosphocholine, LysoPC(17:0), LysoPC(18:0), LysoPC(22:6), LysoPC(18:1), PC(40:8), PC(38:9), PC(38:8), PE(40:8), PC(36:6), PC(38:5), PC(40:9), PC(40:10), DHAP(18:0), PC(36:8), PC(37:6), PC(42:9), PC(37:5), Eicosapentaenoic acid, PC(34:1), LysoPC(18:2), PC(34:4)
FD exposed group	LysoPC(16:0), Glycerophosphocholine, LysoPC(17:0), LysoPC(18:0), LysoPC(22:6), LysoPC(18:1), LysoPC(18:2), Phytosphingosine, PC(38:8), PC(40:8), LysoPA(P-16:0/0:0), PC(40:9), PE(40:8), LysoPC(20:4), Eicosapentaenoic acid, PC(38:5), Dihomo-γ-linolenoylcholine
FI exposed group	LysoPC(16:0), Glycerophosphocholine, LysoPC(22:6), LysoPC(18:1), PC(40:8), LysoPC(17:0), PC(38:8), LysoPC(18:0), PC(40:9), PC(36:4), PC(38:5), PC(37:5), PE(40:8), LysoPC(18:2), LysoPC(20:5), PC(42:9), LysoPC(18:3), LysoPA(P-16:0/0:0), PC(38:9), PC(37:6), PC(34:4), LysoPC(20:4)
FO exposed group	LysoPC(16:0), Glycerophosphocholine, PC(38:9), Plastoquinone 3, PC(40:10), LysoPC(18:0), PC(40:9), LysoPC(17:0), PC(38:8), PC(34:1), PC(40:8), PC(37:6), LysoPC(22:6)
MI exposed group	LysoPC(16:0), Glycerophosphocholine, PC(40:8), LysoPC(22:6), PC(40:9), PC(38:8), LysoPC(18:1), PC(38:9), LysoPC(17:0), PC(42:9), PE(40:8), PC(37:5), PC(34:1), PC(38:5), LysoPC(18:0), PC(37:6), PC(40:10), Phytosphingosine, LysoPC(18:2), Plastoquinone 3, PC(42:10), PC(36:8), LysoPA(P-16:0/0:0), LysoPC(20:5), LysoPC(18:3), Eicosapentaenoic acid

Table S3. The information of the disordered metabolic pathways in zebrafish exposed to fipronil.

Pathway Name	Match Status	p	-log(p)	Holm p	FDR	Impact
Glycerophospholipid metabolism	4/38	6.0227E-6	5.2202	5.0591E-4	5.0591E-4	0.23636
Linoleic acid metabolism	1/4	0.016614	1.7795	1.0	0.6978	0.33333
alpha-Linolenic acid metabolism	1/13	0.053157	1.2744	1.0	1.0	0.125
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	1/13	0.053157	1.2744	1.0	1.0	0.13333
Ether lipid metabolism	1/20	0.080791	1.0926	1.0	1.0	0.0
Arachidonic acid metabolism	1/33	0.13033	0.88496	1.0	1.0	0.03125
Biosynthesis of unsaturated fatty acids	1/35	0.13775	0.86091	1.0	1.0	0.0

Table S4. The information of the disordered metabolic pathways in zebrafish exposed to mixed fiproles.

Pathway Name	Match Status	p	-log(p)	Holm p	FDR	Impact
Glycerophospholipid metabolism	5/38	1.9996E-7	6.699	1.6797E-5	1.6797E-5	0.27272
Linoleic acid metabolism	1/4	0.019363	1.713	1.0	0.81325	0.33333
alpha-Linolenic acid metabolism	1/13	0.061759	1.2093	1.0	1.0	0.125
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	1/13	0.061759	1.2093	1.0	1.0	0.13333
Glycerolipid metabolism	1/16	0.075537	1.1218	1.0	1.0	0.04545
Ether lipid metabolism	1/20	0.093638	1.0285	1.0	1.0	0.0
Sphingolipid metabolism	1/21	0.098115	1.0083	1.0	1.0	0.0
Phosphatidylinositol signaling system	1/28	0.12893	0.88965	1.0	1.0	0.02128
Arachidonic acid metabolism	1/33	0.15038	0.8228	1.0	1.0	0.03125
Biosynthesis of unsaturated fatty acids	1/35	0.15884	0.79905	1.0	1.0	0.0

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