Supplementary Information for

Single-cell Mass Spectrometry with Multi-solvent Extraction Identifies Metabolic Differences between Left and Right Blastomeres in the 8-cell Frog (*Xenopus*) Embryo

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Table S1. Molecular features repeatedly detected in D1R blastomeres in 8-cell *Xenopus* embryos, listed as accurate mass (separation time, min) values. Identified features are in bold (see **Table S2**). Features highlighted in grey mark ions that were used for multivariate and statistical analyses.

146.1655 (10.03)	**89.1072 (10.44)**	221.9824 (12.29)	151.0450 (12.30)	277.0252 (12.30)
230.0960 (12.31)	180.1496 (13.40)	393.9036 (13.55)	214.1337 (13.64)	194.1650 (13.88)
62.0599 (14.07)	144.0479 (14.45)	**265.1115 (14.45)**	**104.1072 (15.20)**	255.1550 (15.33)
246.1562 (15.65)	**234.1448 (15.70)**	**262.1505 (16.02)**	**399.1443 (16.03)**	**133.0971 (16.24)**
147.1126 (16.39)	**90.0545 (16.43)**	**161.1288 (16.52)**	**175.1186 (16.89)**	**104.0705 (17.07)**
189.1596 (17.10)	**156.0766 (17.14)**	**146.1174 (17.78)**	143.1171 (17.78)	**150.1123 (17.79)**
164.1280 (17.79)	184.1120 (17.79)	104.0705 (17.86)	**139.0502 (18.54)**	141.0654 (18.56)
152.0566 (18.79)	261.9759 (18.90)	276.9870 (18.91)	**162.1124 (18.98)**	**139.0502 (19.44)**
141.0654 (19.48)	134.1170 (19.50)	**166.0729 (19.75)**	150.0551 (19.82)	194.1540 (20.18)
204.1227 (20.88)	210.1501 (21.09)	190.1435 (21.21)	174.1273 (21.26)	**218.1389 (21.64)**
76.0393 (21.67)	**148.0600 (21.69)**	226.1445 (21.81)	**132.0774 (21.98)**	208.1543 (22.23)
230.1175 (22.73)	262.1646 (23.77)	**90.0545 (23.89)**	**291.1289 (25.17)**	**118.0861 (27.34)**
132.1024 (27.66)	**106.0501 (27.66)**	425.1888 (27.87)	**132.1024 (28.01)**	**120.0652 (29.43)**
133.0608 (29.45)	**188.0702 (29.99)**	**205.0964 (29.99)**	**150.0589 (30.14)**	**147.0768 (30.25)**
116.0705 (30.33)	**162.0760 (30.35)**	**162.0759 (30.64)**	**159.0759 (30.65)**	**176.1027 (30.68)**
190.1174 (30.75)	**148.0600 (30.78)**	**166.0862 (31.34)**	286.3101 (31.39)	**189.1236 (31.63)**
182.0812 (31.78)	**116.0704 (32.38)**	261.9759 (32.43)	243.9614 (32.53)	**137.0458 (32.59)**
187.1073 (32.82)	**205.1181 (32.95)**	**134.0454 (34.29)**	**118.0860 (35.02)**	**307.0836 (35.72)**
369.0052 (39.14)	**308.0907 (39.15)**			

Table S2. Metabolites identified using single-cell CE-ESI-MS and multi-solvent small-molecule extraction. Unless noted, metabolite signals were singly protonated.

ID	Metabolite (Abbrev.)		Formula	t_m (min)	*m/z* measured	*m/z* theoretical	Δ*m/z* (mDa)	Δ*m/z* (ppm)
1	Spermidine**		$C_7H_{19}N_3$	10.03	146.1655	146.1652	0.3	2.2
2	Putrescine‡		$C_4H_{12}N_2$	10.44	89.1072	89.1073	0.1	1.4
3	Ethanolamine‡	EA	C_2H_7NO	14.07	62.0599	62.0600	0.1	2.3
4	Thiamine**		$C_{12}H_{16}N_4OS$	14.45	265.1115	265.1118	0.3	1.0
5	Choline*,**	Cho	$C_5H_{13}NO$ ($^+$)	15.20	104.1072	104.1070	0.2	2.0
6	Arg-Ala**	RA	$C_9H_{19}N_5O_3$	15.65	246.1562	246.1561	0.1	0.5
7	Lys-Ser**	KS	$C_9H_{19}N_3O_4$	15.70	234.1448	234.1448	0.0	0.1
8	Ser-Arg**	SR	$C_9H_{19}N_5O_4$	16.02	262.1505	262.1510	0.5	1.8
9	S-adenosyl-methionine**	SAM	$C_{15}H_{22}N_6O_5S$	16.03	399.1443	399.1445	0.2	0.5
10	Ornithine**	Orn	$C_5H_{12}N_2O_2$	16.24	133.0971	133.0972	0.1	0.4
11	Lysine*,**	Lys	$C_6H_{14}N_2O_2$	16.39	147.1126	147.1128	0.2	1.4
12	Sarcosine‡	Sar	$C_3H_7NO_2$	16.43	90.0545	90.0550	0.5	5.1
13	Homolysine**		$C_7H_{16}N_2O_2$	16.52	161.1288	161.1285	0.3	2.1
14	Arginine*,**	Arg	$C_6H_{14}N_4O_2$	16.89	175.1186	175.1190	0.4	2.0
15	γ-aminobutyric acid*	GABA	$C_4H_9NO_2$	17.07	104.0705	104.0706	0.1	1.0
16	N6,N6,N6-trimethyl-lysine**	TML	$C_9H_{20}N_2O_2$	17.10	189.1596	189.1598	0.2	0.8
17	Histidine*,**	His	$C_6H_9N_3O_2$	17.14	156.0766	156.0768	0.2	1.0
18	Acetylcholine*,**	AcCho	$C_7H_{15}NO_2$ ($^+$)	17.78	146.1174	146.1176	0.2	1.1
19	Trolamine‡	TEA	$C_6H_{15}NO_3$	17.79	150.1123	150.1125	0.2	1.1
20	Cis-urocanate**	cURA	$C_6H_6N_2O_2$	18.54	139.0502	139.0502	0.0	0.0
21	Guanine**		$C_5H_5N_5O$	18.79	152.0566	152.0567	0.1	0.6
22	Carnitine*,**	Car	$C_7H_{15}NO_3$	18.98	162.1124	162.1125	0.1	0.4
23	Trans-urocanate**	tURA	$C_6H_6N_2O_2$	19.44	139.0502	139.0502	0.0	0.0
24	Methyl guanine**		$C_6H_7N_5O$	19.75	166.0729	166.0723	0.6	3.4
25	Acetylcarnitine*,**	AcCar	$C_9H_{17}NO_4$	20.88	204.1227	204.1230	0.3	1.6
26	Propionylcarnitine**		$C_{10}H_{19}NO_4$	21.64	218.1389	218.1387	0.2	1.0
27	Glycine*	Gly	$C_2H_5NO_2$	21.67	76.0393	76.0393	0.0	0.1
28	N-Methyl-aspartic acid**		$C_5H_9NO_4$	21.69	148.0600	148.0604	0.4	3.0
29	Creatine*,**	CR	$C_4H_9N_3O_2$	21.98	132.0774	132.0768	0.6	5.0
30	Alanine*	Ala	$C_3H_7NO_2$	23.89	90.0545	90.0550	0.5	5.0
31	Arginino-succinate**		$C_{10}H_{18}N_4O_6$	25.17	291.1289	291.1299	1.0	3.5
32	Valine*,**	Val	$C_5H_{11}NO_2$	27.34	118.0861	118.0863	0.2	1.3
33	Isoleucine*	Ile	$C_6H_{13}NO_2$	27.66	132.1024	132.1019	0.5	3.8
34	Serine*,**	Ser	$C_3H_7NO_3$	27.66	106.0501	106.0499	0.2	2.2

ID	Metabolite (Abbrev.)		Formula	t_m (min)	*m/z* measured	*m/z* theoretical	Δ*m/z* (mDa)	Δ*m/z* (ppm)
35	Leucine*	Leu	$C_6H_{13}NO_2$	28.01	132.1024	132.1019	0.5	3.8
36	Threonine*,**	Thr	$C_4H_9NO_3$	29.43	120.0652	120.0655	0.3	2.7
37	Asparagine*,**	Asn	$C_4H_8N_2O_3$	29.45	133.0608	133.0608	0.0	0.2
38	Tryptophan*,**	Trp	$C_{11}H_{12}N_2O_2$	29.99	205.0964	205.0972	4.7	3.7
39	Methionine*,**	Met	$C_5H_{11}NO_2S$	30.14	150.0589	150.0583	0.6	3.8
40	Glutamine*,**	Gln	$C_5H_{10}N_2O_3$	30.25	147.0768	147.0764	0.4	2.6
41	2-Aminoadipic acid**		$C_6H_{11}NO_4$	30.35	162.0760	162.0761	0.1	0.5
42	Acetylhomoserine†		C6H11NO4	30.64	162.0759	162.0761	0.2	1.1
43	Citrulline**	Cit	$C_6H_{13}N_3O_3$	30.68	176.1027	176.1030	0.3	1.5
44	Homocitrulline**		$C_7H_{15}N_3O_3$	30.75	190.1174	190.1186	1.2	6.4
45	Glutamic acid*,**	Glu	$C_5H_9NO_4$	30.78	148.0600	148.0604	0.4	2.9
46	Phenylalanine*,**	Phe	$C_9H_{11}NO_2$	31.34	166.0862	166.0863	0.1	0.3
47	Acetyllysine†		$C_8H_{16}N_2O_3$	31.63	189.1236	189.1234	0.2	1.2
48	Tyrosine*,**	Tyr	$C_9H_{11}NO_3$	31.78	182.0812	182.0812	0.0	0.2
49	Proline*,**	Pro	$C_5H_9NO_2$	32.38	116.0704	116.0706	0.2	1.8
50	Hypoxanthine**	HPX	$C_5H_4N_4O$	32.59	137.0458	137.0458	0.0	0.1
51	Ser-Val†	SV	$C_8H_{16}N_2O_4$	32.95	205.1181	205.1183	0.2	0.9
52	Aspartic acid*,**	Asp	$C_4H_7NO_4$	34.29	134.0454	134.0448	0.6	4.6
53	Glycine betaine*	GB	$C_5H_{11}NO_2$	35.02	118.0860	118.0863	0.3	2.2
54	Glutathione, oxidized**	GSSG	$C_{20}H_{32}N_6O_{12}S_2(^{2+})$	35.72	307.0836	307.0833	0.3	1.1
55	Glutathione*,**	GSH	$C_{10}H_{17}N_3O_6S$	39.15	308.0907	308.0911	0.4	1.2

Note: Asterisk (*) signifies identifications based on migration-time comparison against related chemical standards. Double asterisk (**) denotes identifications supplemented by tandem mass spectrometry experiments performed on related standards or data available in Metlin (https://metlin.scripps.edu/), Human metabolome database (http://www.hmdb.ca/), or mzCloud (https://www.mzcloud.org/), last accessed on Jan. 25, 2016. Dagger (†) indicates that the tandem mass spectrum agrees with fragmentation predicted in Mass Frontier 7.0 (Thermo Scientific). Double dagger (‡) indicates most probable assignment for endogenous metabolite based on mass-matches in Metlin (5 ppm accuracy).

Table S3. Fisher's least significant difference (LSD) analysis revealing differential metabolite extraction between $polar_{pH4}$, $apolar_{pH5}$, and $apolar_{pH8}$ solvents.

Metabolite	*p* value	Fisher's LSD
Methionine	0.0006	$Apolar_{pH5}$–$Apolar_{pH8}$; $Apolar_{pH5}$–$Polar_{pH4}$
Glutamate	0.0003	$Apolar_{pH5}$–$Apolar_{pH8}$; $Apolar_{pH5}$–$Polar_{pH4}$
Glutamine	0.0278	$Apolar_{pH5}$–$Polar_{pH4}$; $Apolar_{pH5}$–$Apolar_{pH8}$
SAM	0.0077	$Apolar_{pH5}$–$Polar_{pH4}$
Citrulline	0.0163	$Apolar_{pH5}$–$Polar_{pH4}$
Alanine	0.0322	$Apolar_{pH8}$–$Polar_{pH4}$; $Apolar_{pH8}$–$Apolar_{pH5}$
Ornithine	0.0178	$Apolar_{pH8}$–$Polar_{pH4}$; $Apolar_{pH8}$–$Apolar_{pH5}$
Valine	0.0038	$Apolar_{pH8}$–$Polar_{pH4}$; $Apolar_{pH5}$–$Polar_{pH4}$
Threonine	0.0074	$Apolar_{pH8}$–$Polar_{pH4}$; $Apolar_{pH5}$–$Polar_{pH4}$
GSSG	0.0225	$Apolar_{pH8}$–$Apolar_{pH5}$; $Polar_{pH4}$–$Apolar_{pH5}$
GB	0.0190	$Apolar_{pH8}$–$Polar_{pH4}$
GSH	0.0081	$Polar_{pH4}$–$Apolar_{pH8}$; $Polar_{pH4}$–$Apolar_{pH5}$
HPX	0.0077	$Polar_{pH4}$–$Apolar_{pH8}$; $Apolar_{pH5}$–$Apolar_{pH8}$

Table S4. Differentially enriched metabolites between D1L and D1R blastomeres in the 8-cell *Xenopus* embryo. Fold change (FC) *vs. p* value were calculated based on normalized peak areas and filtered for statistical and biological significance, $p < 0.05$ and FC ≥ 1.5 (see also **Fig. 4A**). Accurate mass and migration time (MT) values are provided for small molecules that are yet to be identified.

Small Molecule *m/z* (MT, min)	FC	$\log_2$(FC)	*p*	$-10\times\log_{10}(p)$	Solvent
Acetylcarnitine	0.539	–0.891	0.002104	26.77	$Apolar_{pH5}$
Creatine	0.387	–1.369	0.001198	29.22	$Apolar_{pH5}$
Putrescine	0.487	–1.038	0.017126	17.66	$Apolar_{pH5}$
Spermidine	0.035	–4.852	0.000704	31.52	$Apolar_{pH5}$
SAM	0.602	–0.732	0.000581	32.36	$Polar_{pH4}$
255.1550 (15.32)	0.140	–2.834	3.36E-05	44.73	$Apolar_{pH5}$
285.1664 (15.92)	0.208	–2.266	0.000807	30.93	$Apolar_{pH5}$
106.0860 (17.1)	0.224	–2.157	2.8E-05	45.52	$Apolar_{pH5}$
186.1125 (20.54)	0.167	–2.579	8.08E-06	50.93	$Apolar_{pH5}$
172.0943 (20.65)	0.077	–3.702	0.019707	17.05	$Apolar_{pH5}$
104.0705 (23.98)	0.257	–1.957	0.036122	14.42	$Apolar_{pH5}$
262.1646 (24.05)	0.373	–1.423	0.014958	18.25	$Apolar_{pH5}$
137.0297 (12.41)	0.137	–2.867	0.005169	22.87	$Apolar_{pH8}$
400.6067 (13.3)	0.230	–2.118	0.029715	15.27	$Apolar_{pH8}$
90.0550 (16.43)	0.195	–2.355	0.001344	28.72	$Polar_{pH4}$
106.0860 (17.13)	0.433	–1.209	0.03467	14.60	$Polar_{pH4}$
162.1127 (19.22)	0.202	–2.309	0.000224	36.50	$Polar_{pH4}$
138.0552 (30.93)	0.239	–2.064	0.028664	15.43	$Polar_{pH4}$
Ethanolamine	1.518	0.602	0.009845	20.07	$Apolar_{pH5}$
GABA	3.095	1.630	0.007152	21.46	$Apolar_{pH5}$
GABA	2.968	1.569	0.012432	19.05	$Apolar_{pH8}$
Isoleucine	2.079	1.056	0.023224	16.34	$Apolar_{pH5}$
Leucine	2.411	1.269	0.029559	15.29	$Apolar_{pH5}$
Trolamine	1.989	0.992	0.000835	30.78	$Polar_{pH4}$
75.0755 (14.62)	8.500	3.087	0.001058	29.76	$Apolar_{pH5}$
170.0962 (17.78)	1.725	0.786	0.039921	13.99	$Apolar_{pH5}$
106.0860 (18.15)	5.320	2.411	0.032642	14.86	$Apolar_{pH5}$
184.1120 (18.15)	1.856	0.892	0.043493	13.62	$Apolar_{pH5}$
425.1888 (27.64)	2.814	1.492	0.035694	14.47	$Apolar_{pH5}$
138.0552 (30.92)	1.632	0.706	0.017684	17.52	$Apolar_{pH5}$
180.1496 (13.45)	1.520	0.604	0.016443	17.84	$Apolar_{pH8}$
150.0551 (19.82)	1.570	0.651	0.038984	14.09	$Apolar_{pH8}$
146.1176 (16.52)	4.436	2.149	0.012419	19.06	$Apolar_{pH8}$
144.0479 (14.45)	1.821	0.865	0.018954	17.22	$Apolar_{pH8}$
138.0552 (23.58)	1.629	0.704	0.023697	16.25	$Apolar_{pH8}$

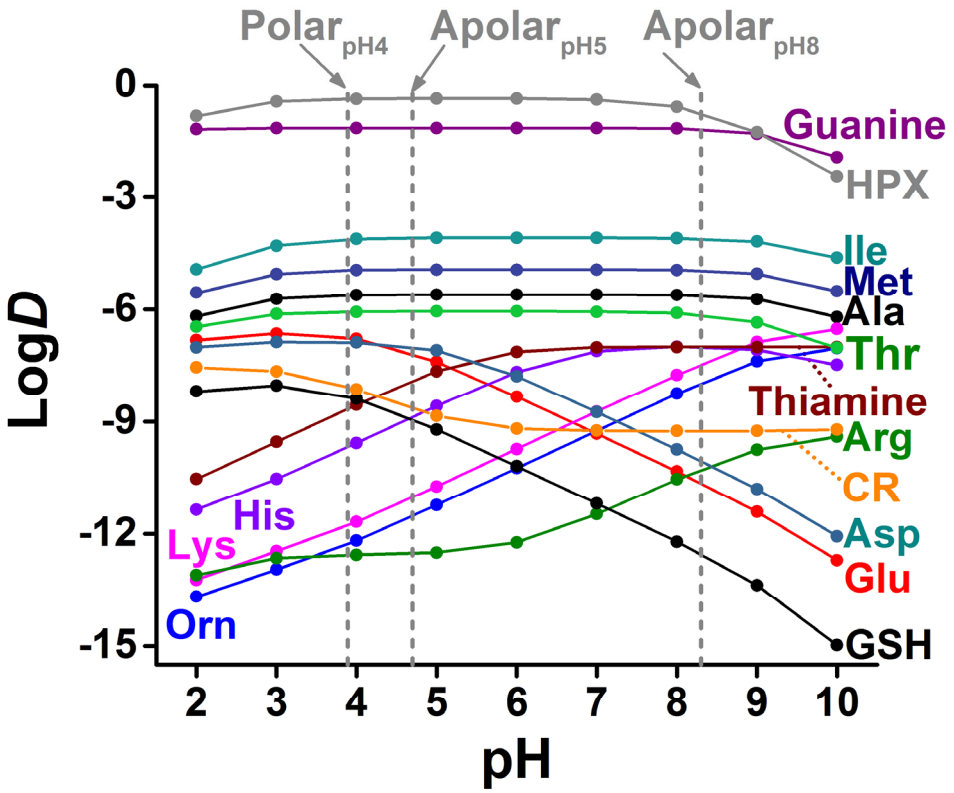

Figure S1. Octanol/water distribution coefficients (*D*) calculated as a function of pH. To facilitate the detection of metabolites, extraction solvents with complementary polarity and pH characteristics were used in this study: 50% (v/v) methanol with pH 3.89 ($polar_{pH4}$); 40% (v/v) acetonitrile and 40% (v/v) methanol with pH 4.7 ($apolar_{pH5}$); 40% (v/v) acetonitrile and 40% (v/v) methanol with pH 8.3 ($apolar_{pH8}$). Key: CR, creatine; HPX, hypoxanthine; GSH, glutathione.

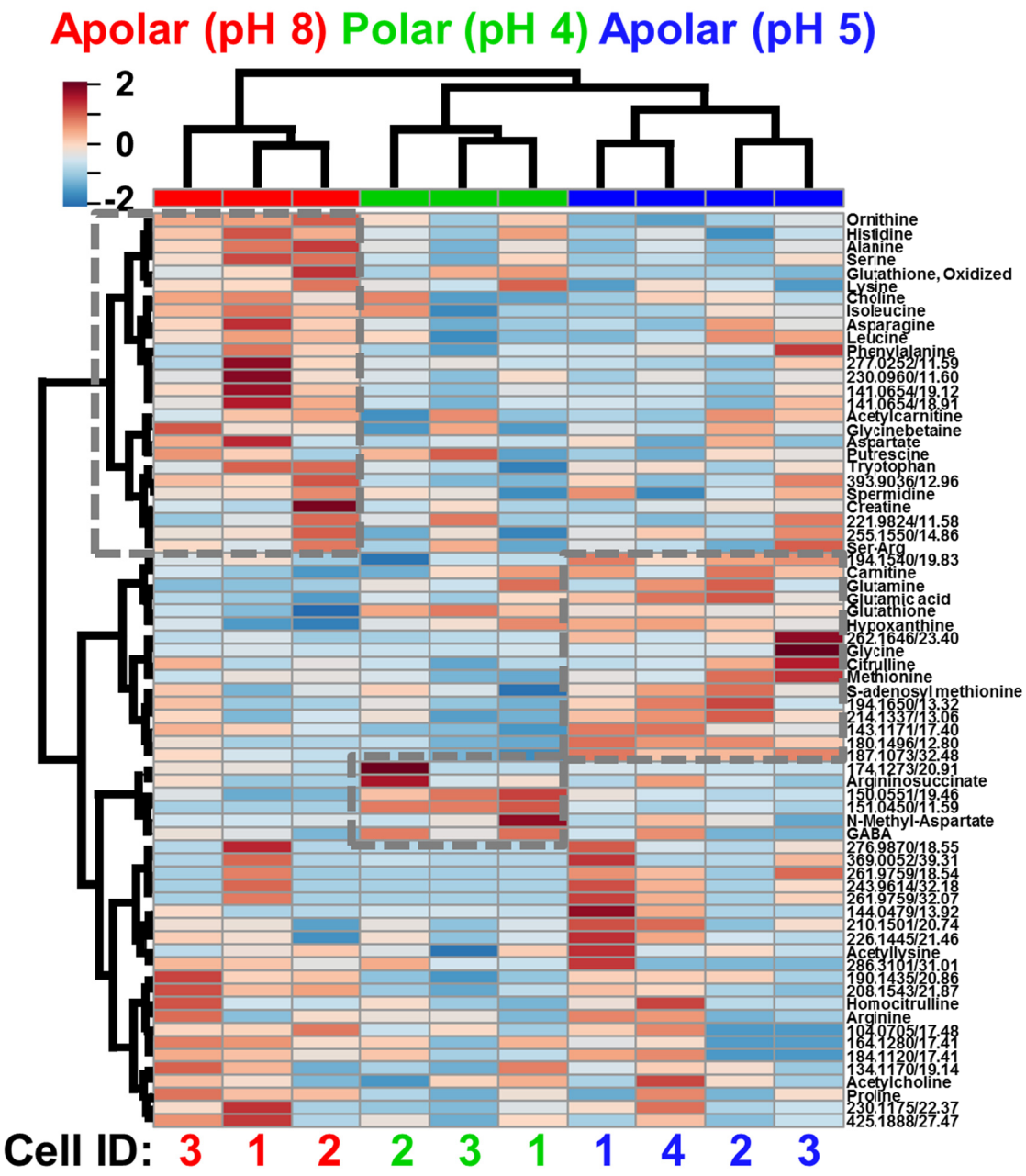

Figure S2. HCA-heatmap showing differential metabolite extraction from single D1R blastomeres using the three solvents. Individual blastomeres are labeled using their unique identifier (see Cell ID). Small molecules that have yet to be identified are labeled with accurate mass (*m/z* value)/migration time (min) information.