Supplemental Information

Comparability of Liquid Chromatography Tandem Mass Spectrometry Analysis of Dissolved Organic Matter Across Laboratories

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Supplemental Methods: Global Data Analysis Procedure

Raw data was converted to .mzML format using msConvert. Feature finding for Feature-Based Molecular Networking (FBMN) was accomplished with mzmine (ver. 4.2.0). Mass detection thresholds of 1E5 for MS1 and 1E4 for MS2 were employed. The Chromatogram builder module was used to assemble chromatograms with a minimum of 3 consecutive scans, minimum intensity of 5E5 for consecutive scans, a minimum height of 1E6 and an *m/z* tolerance of 5 ppm. This was followed by the Local minimum feature resolver with a chromatographic threshold of 10%, a minimum search range of 0.1 min, a minimum absolute height of 1E6, a minimum peak top/edge ratio of 1.4, a peak duration range of 0.05 to 3 min and a minimum of 5 scans, while MS/MS scans were paired using the feature edges, *m/z* tolerances of 0.005 Da or 10 ppm and a minimum relative height of 25%. The 13C isotope filter was used with an *m/z* tolerance of 5 ppm and a retention time tolerance of 0.1 min and a maximum charge of 2. Individual feature lists were aligned with the Join aligner using an *m/z* tolerance of 5 ppm and a retention time tolerance of 1.7 min (weights = 3/1). The aligned feature list was filtered to contain only features that were contained in at least 10 samples and had an assigned MS/MS spectrum. Duplicate features were merged using the Duplicate peak filter module with the NEW AVERAGE mode, an *m/z* tolerance of 5 ppm and a retention time tolerance of 1.7 min. The final feature list and associated MS/MS spectra file were then exported and uploaded to GNPS2. The files were analyzed through the FBMN workflow using precursor and fragment ion tolerances of 0.02. Window and precursor window filters were activated, molecular networking was carried out with a minimum cosine of 0.7 and a minimum of 6 matched fragments. Network topology parameters were set to 10 for Top K and 100 for the maximum component size. Library search parameters were set to a minimum cosine of 0.7 and a minimum of 4 matched fragments. The same parameters were used for both ionization modes. The ESI+ FBMN job can be accessed at: https://gnps2.org/status?task=61e48c4af96944aeba73f59f0dbd51c2. The ESI- FBMN can be accessed at: https://gnps2.org/status?task=88f8fdb9665b42688b53cf560f4e23fd.

Classical Molecular Networking (CMN) was carried out using precursor and fragment ion tolerances of 0.02 Da. Window and precursor window filters were activated, molecular networking was carried out with a minimum cosine of 0.7, a minimum of 6 matched fragments and a minimum cluster size of 10. Network topology parameters were set to 10 for Top K and 100 for the maximum component size. Library search parameters were set to a minimum cosine of 0.7 and a minimum of 4 matched fragments. The ESI+ CMN job can be accessed at https://gnps2.org/status?task=cc2c071be92f428ca85188ae3654ea32 and the ESI- CMN job at https://gnps2.org/status?task=b8ad0450cf134dca9932394f856125f1.
Upset plots were generated with Intervene (https://asntech.shinyapps.io/intervene/) (Khan & Mathelier, 2017) and blank removal, PCoA plots and Random Forest Analysis (fixed random seed and 100 trees) were carried out using the FBMN-STATS APP (https://fbmn-statsguide.gnps2.org/; Kelminal et al., 2024) where data was generally imputed and normalized (TIC normalization). LC-MS/MS heatmaps and extracted ion chromatograms (XICs) were plotted using the GNPS dashboard (https://dashboard.gnps2.org/). All other plots and merging of feature tables based on identical matched library IDs were carried out using dedicated python scripts. Processed and

source files as well as python scripts can be accessed at ZENODO https://doi.org/10.5281/zenodo.16897529.

Supplemental Tables

Table S1: Final concentrations of study samples

Sample	A	A45M	A15M	A5M	M
Marine DOM [µg/mL]	0	4500	4500	4500	4500
Algae Extract [µg/mL]	225	225	75	25	0.0
Internal Standards [µg/mL]	0	0.12	0.12	0.12	0.12

Table S2: Exact masses of internal standards

Compound	Molecular Formula	Mass $[M+H]^+$	Mass $[M+Na]^+$
Domoic acid	$C_{15}H_{21}NO_6$	312.1442	334.1261
Kainic acid	$C_{10}H_{15}NO_4$	214.1074	236.0893
Isoxaben	$C_{18}H_{24}N_2O_4$	333.1809	355.1628
Irgarol	$C_{11}H_{19}N_5S$	254.1434	276.1253
Imazapyr	$C_{13}H_{15}N_3O_3$	262.1186	284.1006
Heroin	$C_{21}H_{23}NO_5$	370.1649	392.1468
Methamphetamine	$C_{10}H_{15}N$	150.1277	172.1097
Cocaine	$C_{17}H_{21}NO_4$	304.1543	326.1363

Table S3: Overview of LC methods

Lab	LC Type	Column	Column Dimesions [mm]	Particle Size [µm]	Flow rate [mL/min]	Gradient Length [min]	Total Method length [min]	Gradient	Injection Volume [uL]
a	UHPLC	Phenomenex Kinetex C18	2.0 x 150	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	10
b	UHPLC	Phenomenex Kinetex C18	2.0 x 150	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
p	UHPLC	Phenomenex Kinetex C18	2.0 x 150	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
c	UHPLC	Waters Acquity HSS T3	2.1 x 100	1.8	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
d	UHPLC	Waters Acquity BEH C18	2.1 x 150	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
e	UHPLC	Phenomenex Kinetex C18	2.1 x 150	1.7	0.5	10	18	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (17min), 5% (18Min)	5
f	UHPLC	Waters Acquity UPLC CSH C18	2.1 x 150	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
g	UHPLC	Waters Acquity UPLC BEH C18	2.1 x 100	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
q	UHPLC	Waters Cortecs C18	2.1 x 150	1.6	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
h	HPLC	Hamilton PRP-C18	2.0 x 150	5	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
r	UHPLC	Thermo Raptor C18	2.1 x 150	1.8	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
i	UHPLC	Phenomenex Kinetex C18	2.1 x 150	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
j	UHPLC	Phenomenex Kinetex C18	2.1 x 100	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
s	UHPLC	Thermo Hypersil Gold aq	2.1 x 100	1.9	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
k	UHPLC	Thermo Hypersil Gold C18	2.1 x 150	1.9	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
l	UHPLC	Phenomenex Kinetex C18	2.1 x 100	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
t	UHPLC	Waters Acquity BEH C18	2.1 x 100	1.7	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
m	HPLC	Grace Genesis C18	2.1 x 150	3	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	10
u	UHPLC	Waters Acquity HSS T3	3 x 150	1.8	0.4	14	17	5% to 50% B (8 min), 50% to 99% B (11 min), 99% to 99% B (14min), 5% to 5% B (17Min)	5
v	Nano-LC	Thermo PepMap C18	0.05 x 150	2	0	15	40	10% to 95% (15 min)	1
n	UHPLC	Thermo Hypersil Gold	2.1 x 100	1.9	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
o	UHPLC	Thermo Hypersil Gold C18	2.1 x 100	1.9	0.4	10	17	5% to 50% B (7 min), 50% to 99% B (10 min), 99% to 99% B (13min), 5% to 5% B (17Min)	5
w	UHPLC	Phenomenex Kinetex C18	2.1 x 150	1.7	0.4	14	18	5% to 50% B (8 min), 50% to 99% B (11 min), 99% to 99% B (14min), 5% to 5% B (18Min)	10
x	UHPLC	Waters Cortecs C18	2.1 x 150	1.6	0.4	12	16	5% to 50% B (9 min), 50% to 95% B (11 min), 95% to 99% B (11.5 min), 95% to 5% B (14.5 min), 5% to 5% B (15.5 min)	5

Table S4: Overview of MS methods

Lab	Mass Spectrometer	Mass Range	MS1 Resolution	MS2 Resolution	Micro Scans	Max Fill Time MS1	Max Fill Time MS2	AGC MS1	AGC MS2	Isolation Width [m/z]	Collision Energy	Apex Trigger Range	Minimum AGC MS2	MS/MS Threshold Relative (optional)	MS/MS Threshold Absolute	TopN DDA	Dynamic Exclusion [sec]	DDA Duty Cycle Time [sec]
a	Q-Exactive	150-1500	70000	17500	1	100	150	1.0E+06	1.0E+06	1	20,30,40	2-15	3.0E+04	3%	2.0E+05	5	5	<1
b	Q-Exactive	150-1500	70000	17500	1	100	150	1.0E+06	1.0E+06	1	20,30,40	2-15	3.0E+04	N/A	N/A	5	5	<1
p	LTQ-Orbitrap	150-1500	120000	15000	1	100	100	1.0E+06	5.0E+04	N/A	35	N/A	2.5E+03	N/A	N/A	2	5	~1
c	Orbitrap Fusion Lumos	150-1500	120000	30000	1	100	100	default	default	N/A	20,30,40	N/A	N/A	N/A	2.5E+04	5	5	~1
d	Orbitrap IDX	150-1500	120000	30000	1	256	100	2.0E+05	5.0E+04	N/A	20,30,40	3	N/A	N/A	1.0E+05	5	5	<1
e	Q-Exactive	150-1500	140000	17500	1	100	150	1.0E+06	1.0E+05	N/A	20,30,40	2-15	8.0E+03	N/A	5.3E+04	5	5	<1
f	Q-Exactive HF	150-1500	60000	30000	1	100	150	1.0E+06	1.0E+05	N/A	20,30,40	2-15	8.0E+03	N/A	5.3E+04	5	5	<1
g	Q-Exactive+	150-1500	140000	35000	1	50	100	1.0E+06	1.0E+05	1	20,30,40	2-15	8.0E+03	N/A	1.6E+05	5	5	<1
q	QTOF, maXis	150-1500	40000	40000		100	200	N/A	N/A	4	35	N/A	N/A	N/A	2.0E+03	5	5	~1
h	Q-Exactive	150-1500	70000	17500	1	100	150	1.0E+06	1.0E+05	N/A	20,30,40	2-15	3.0E+04	N/A	2.0E+05	5	10	~1
r	Orbitrap ID-X	150-1500	120000	30000	1	100	100	default	default	N/A	20,30,40			N/A	2.5E+04	5	5	~1
i	Q-Exactive HF	150-1500	60000	30000	1	100	200	3.0E+06	1.0E+05	1	20,30,40	2-15	8.0E+03	N/A	4.0E+04	5	5	~1
j	Q-Exactive	150-1500	70000	17500	1	100	150	3.0E+06	1.0E+05	N/A	20,30,40	2-15	5.0E+03	N/A	1.6E+05	5	5	<1
s	QTOF, Synapt g2	50-1500	40000	20000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
k	Q-Exactive HF-X	150-1500	120000	30000	1	100	200	3.0E+06	1.0E+05	N/A	20,30,40	2-15	8.0E+03	N/A	4.0E+04	5	5	~1
l	Orbitrap Exploris 120	150-1500	120000	60000	1	100	150	1.0E+06	1.0E+05	N/A	20,30,40	30%	N/A	N/A	2.0E+05	3	5	~1
t	Orbitrap Fusion	150-1500	120000	30000	1	100	200	5.0E+05	2.5E+04	N/A	20,30,40		N/A	N/A	2.5E+04	5	5	~1
m	Q-Exactive	150-1500	140000	35000	1	100	150	3.0E+06	1.0E+05	N/A	20,30,40	2-15	8.0E+03	N/A	5.3E+04	5	5	<1
u	SolariX xR, 12T	147.5-150	512000	512000	N/A	100	400	N/A	N/A	5	10 V	N/A	N/A	5%	N/A	N/A	5	N/A
v	Q-Exactive HF	100-1500	240000	30000	1	100	100	1000000	100000	1	20,30,40	6-12	1.0E+03	0.1	1.0E+04	N/A	20	~1
n	Orbitrap Exploris 480	150-1500	120000	30000	1	100	100	1.0E+06	1.0E+05	1	20,30,40	30%	N/A	N/A	5.0E+03	5	5	~1
o	Q-Exactive	150-1500	70000	17500	1	100	150	1.0E+06	1.0E+05		20,30,40	2-15	1.0E+04	N/A	6.7E+04	5	5	~1
w	Orbitrap Fusion Lumos	150-1500	240000	30000	1	100	100	2.00E+05	5.00E+04	0.8	20,30,40	N/A	N/A	20	2.5E+04	dynamic	exclusion list	1
x	QTOF, X500R	50-1600	42000	27000	N/A	N/A	N/A	N/A	N/A	1	30	N/A	N/A	N/A	500	10	6	0.6

Table S5: Summary of molecular networking metrics from all datasets. Metrics include the presence or absence (✓/✗) of internal standard annotation during classical molecular networking (CMN), as well as the number of library ID matches, clustered features (clusters), networked nodes, singletons, and annotation rates obtained from both CMN and feature-based molecular networking (FBMN).

											CMN+			FBMN+			CMN-			FBMN-		
Lab	Instrument Platform	Annotated Standards	Kainic acid	Imazapyr	Cocaine	Domoic acid	Isoxaben	Heroin	Irgarol	Methamphetamine	Lib IDs	Clusters	Networked Nodes	Lib Ids	Nodes	Networked Nodes	Lib IDs	Clusters	Networked Nodes	Lib Ids	Nodes	Networked Nodes
i	Q-Exactive HF	6	✓	✓	✓	✓	×	✓	✓	×	158	2677	1114	264	1827	1103	54	2153	842	4	447	201
j	Q-Exactive Plus	8	✓	✓	✓	✓	✓	✓	✓	✓	145	2937	1687	577	3112	2441	40	2012	1283	17	1076	551
o	Q-Exactive	6	×	✓	✓	✓	✓	×	✓	✓	136	5061	2760	573	4569	3088	26	2228	1331	8	1071	679
g	Q-Exactive Plus	8	✓	✓	✓	✓	✓	✓	✓	✓	122	2602	1457	401	2431	1755	45	1770	1039	10	648	346
k	Q-Exactive HFX	7	×	✓	✓	✓	✓	✓	✓	✓	106	5058	2776	340	4624	3414	68	3626	1513	9	1922	915
b	Q-Exactive	8	✓	✓	✓	✓	✓	✓	✓	✓	106	2451	1502	187	1789	1267	16	526	328	3	101	24
e	Q-Exactive	7	✓	✓	✓	✓	✓	×	✓	✓	101	2699	1378	348	2401	1662	26	2052	951	8	693	377
f	Q-Exactive HF	6	×	✓	✓	✓	×	✓	✓	✓	101	2958	1244	293	2938	1506	40	1541	593	20	520	179
l	Orbitrap Exploris 120	6	✓	✓	✓	✓	×	×	✓	✓	99	2625	1609	354	2342	1743	38	1998	1194	12	811	551
c	Orbitrap Fusion Lumos	7	✓	✓	✓	✓	✓	×	✓	✓	98	4003	1995	389	2756	1934	45	2555	1251	8	445	271
m	Q-Exactive	7	✓	✓	✓	✓	✓	×	✓	✓	90	1375	723	183	987	657	10	570	140	3	301	24
n	Orbitrap Exploris 480	7	✓	✓	✓	✓	✓	✓	×	✓	86	2394	1180	306	1962	1363	3	1922	914	4	181	85
d	Orbitrap IDX	8	✓	✓	✓	✓	✓	✓	✓	✓	83	3771	1866	423	3520	2370	25	3351	1690	13	1026	686
h	Q-Exactive	7	×	✓	✓	✓	✓	✓	✓	✓	74	2428	1545	175	1304	971	33	1916	1213	11	558	295
a	Q-Exactive	7	✓	✓	✓	×	✓	✓	✓	✓	71	2217	1121	275	1962	1243	24	820	364	8	496	234
s	Synapt G2	0	×	×	×	×	×	×	×	×	3	136	72	0	0	0	12	117	87	**NA**	**NA**	**NA**
p	LTQ-Orbitrap Elite	4	×	✓	✓	✓	×	×	×	✓	7	210	36	2	777	26	30	1082	206	0	174	0
q	QTOF maXis	5	×	✓	✓	×	✓	✓	✓	×	18	604	310	6	948	46	0	45	0	**NA**	**NA**	**NA**
t	Orbitrap Fusion	5	✓	✓	✓	×	✓	✓	×	×	91	2632	1325	202	1996	1394	48	1956	933	3	148	89
u	FT-ICR	0	×	×	×	×	×	×	×	×	0	3741	294	0	57	2	2	484	421	**NA**	**NA**	**NA**
v	Q-Exactive HF	5	✓	✓	✓	✓	×	×	×	✓	107	4262	1969	464	3999	2185	65	4235	2100	8	952	443
w	Orbitrap Fusion Lumos	5	×	✓	✓	✓	✓	✓	×	×	69	501	299	79	288	213	36	712	445	14	149	113
x	QTOF X500R	8	✓	✓	✓	✓	✓	✓	✓	✓	61	3460	789	5	757	5	35	1983	381	2	146	23
r	Orbitrap ID-X	0	×	×	×	×	×	×	×	×	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	22	1835	819	8	176	102

Table S6: Important Metabolites for Sample Classification according to sample-set wise Random Forest Analyses.

Annotation	ID	count	rank	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o
dihydroactinidiolide	26	15	1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
cocamidopropyl betaine	228	15	2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Cabrillostatin	85	15	3	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
6-Methoxyluteolin	54	15	4	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
METHYL STEARATE	138	15	5	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Imazapic	116	15	6	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Melibiose	144	15	7	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
tris(2-butoxyethyl) phosphate	274	14	8	x	x	x		x	x	x	x	x	x	x	x	x	x	x
1-Myristoyl-sn-glycero-3-phosphocholine	16	13	9	x	x	x	x	x		x	x	x	x	x	x		x	x
Loliolide	132	14	10	x	x	x	x		x	x	x	x	x	x	x	x	x	x
triphenyl phosphate	273	12	11	x	x		x	x	x	x	x	x	x	x			x	x
Methyl hexadecanoate	146	13	12		x	x	x	x	x	x	x	x	x	x	x		x	x
Morphine	152	14	13	x	x	x	x	x	x	x	x	x	x	x	x		x	x
Dimethyl sebacate	99	12	14	x			x	x	x	x	x		x	x	x	x	x	x
1-Hexadecanoyl-sn-glycerol	15	15	15	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Monoolein	150	11	16		x		x	x	x	x	x		x	x	x		x	x
Glu Phe	104	14	17	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Benzyldodecyldimethylammonium	81	15	18	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
1,3-Diphenylguanidine	12	13	19	x	x	x	x	x	x		x	x	x	x	x		x	x
DEET	96	14	20	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Monopalmitolein (9c)	151	15	21	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
7512-17-6	57	13	22	x	x	x	x	x	x	x	x		x	x		x	x	x
2,6-Dimethoxy-4-methylphenol	30	13	23	x	x	x	x	x	x	x	x		x	x	x	x	x	
delorazepam	231	10	24	x				x	x		x	x	x	x	x		x	x
GLYCERO-3-PHOSPHOCHOLINE	103	13	25	x	x	x	x		x	x	x	x	x	x	x	x		x
myristamidpropyl betaine	252	12	26	x	x	x	x	x	x	x	x	x	x			x	x	
L-Tryptophan	122	13	27	x	x	x	x	x	x	x	x	x	x		x		x	x
Shinorine	196	14	28	x	x	x	x	x	x	x		x	x	x	x	x	x	x
Phenylalanine methyl ester	179	15	29	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
diethyl phthalate	234	13	30			x	x	x	x	x	x	x	x	x	x	x	x	x
dihydrocapsaicin	237	8	31	x				x		x		x		x	x	x	x	
1-Palmitoylglycerophosphocholine	18	10	32	x	x	x	x			x	x	x	x	x				x
Phe-ala	178	13	33	x	x	x	x	x	x	x	x		x	x	x		x	x
Hexaethylene glycol	111	13	34	x	x	x	x	x	x	x	x	x			x	x	x	x
triethyl citrate	272	10	35	x	x	x		x	x	x	x				x		x	x
3.10S-Hydroxypheophorbide a	39	10	36	x	x		x	x	x	x			x	x	x			x
4-Methylphthalic anhydride	44	12	37	x		x	x	x	x		x	x	x	x	x	x	x	
ibuprofen	243	12	38	x	x	x	x	x			x	x	x		x	x	x	x
laurolactam	246	12	39	x	x	x	x	x	x	x	x	x	x	x			x	
sultiame	265	11	40	x			x	x	x		x	x	x	x	x		x	x
Sebacic acid monomethyl ester	193	10	41				x	x		x	x		x	x	x	x	x	x
Ile-Glu	115	12	42	x	x	x	x	x	x		x	x		x	x	x	x	
benzyltetradecyldimethyl ammonium	218	11	43	x	x	x	x	x	x	x	x			x		x	x	
4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine	43	13	44	x	x	x	x	x	x	x	x	x		x	x		x	x
9,12-Octadecadiynoic Acid	61	13	45	x	x	x	x	x	x	x		x	x	x	x		x	x
Pentapropylene glycol	175	13	46	x	x	x		x	x	x	x	x		x	x	x	x	x
PHENYLALANINE	168	12	47	x	x	x	x	x	x	x	x			x	x	x		x
Val-Leu	210	11	48			x	x	x	x	x	x		x	x	x		x	x
Palythine	171	12	49	x	x		x	x		x	x	x	x	x	x	x	x	
Dioctyl phthalate	100	11	50	x		x	x	x	x			x	x	x	x		x	x
N-[3-(dimethylamino)propyl]dodecanamide	157	12	51		x	x	x	x	x	x	x		x	x	x		x	x
cis-9-Hexadecenoic acid	226	12	52	x	x	x		x	x		x	x	x	x	x		x	x
Chicoric acid	86	13	53	x			x	x	x	x	x	x	x	x	x	x	x	x
domoic acid	240	13	54	x	x	x	x	x	x	x	x	x		x	x		x	x
Methyl-Domoic Acid	147	12	55	x	x	x	x	x	x	x	x	x			x	x	x	
B08A23	77	11	56	x	x	x	x		x		x		x	x	x		x	x
diphenyl phosphate	239	9	57	x	x		x	x	x	x			x		x		x	
SQDG(16:0/16:1)	189	10	58	x			x		x		x	x	x		x	x	x	x
Tri(propylene glycol) butyl ether	204	13	59	x	x	x	x	x	x	x	x		x	x	x		x	x
Vanillin	211	9	60	x		x	x	x			x		x	x	x			x

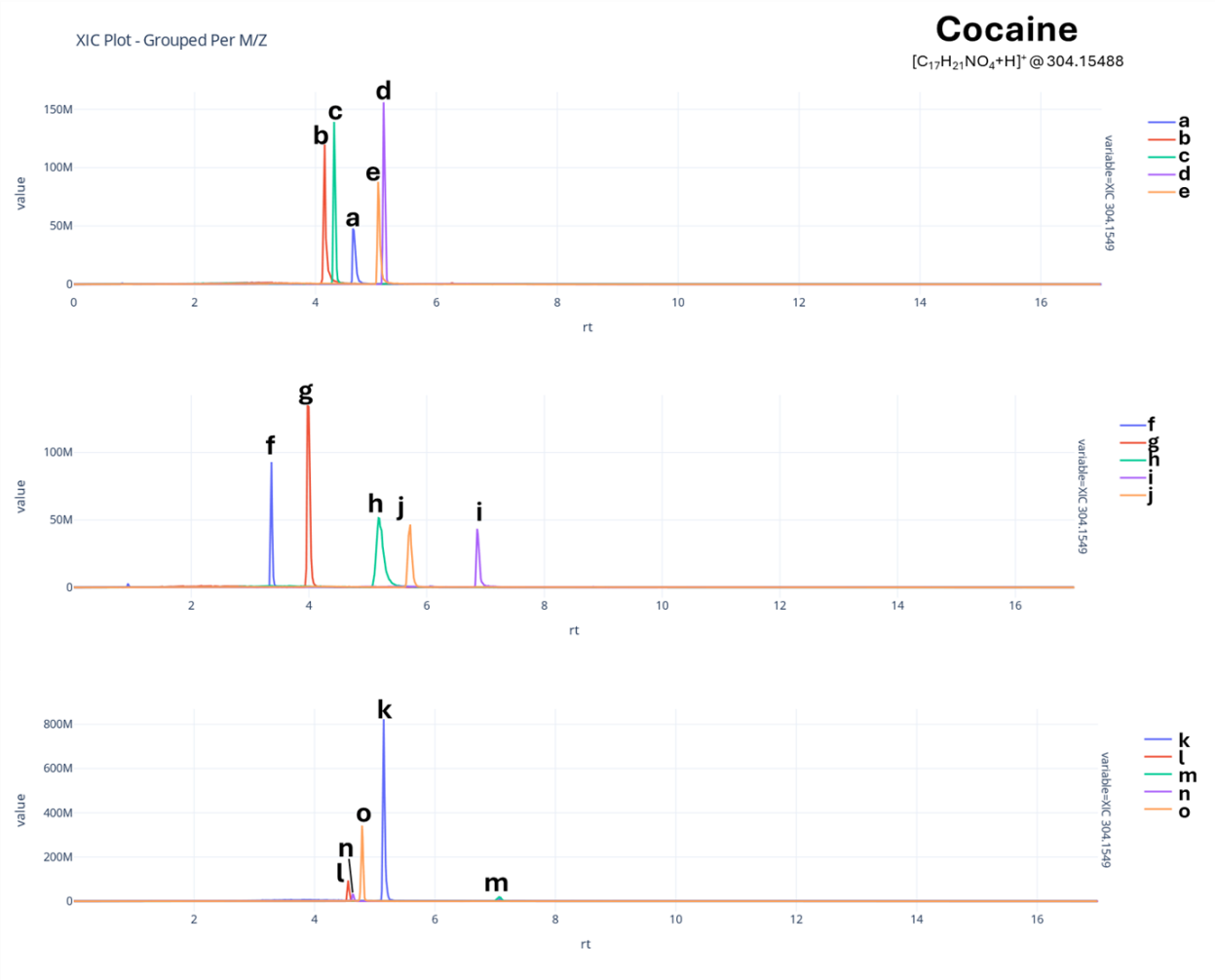

Figure S1: XICs of Cocaine for the 15 selected datasets.

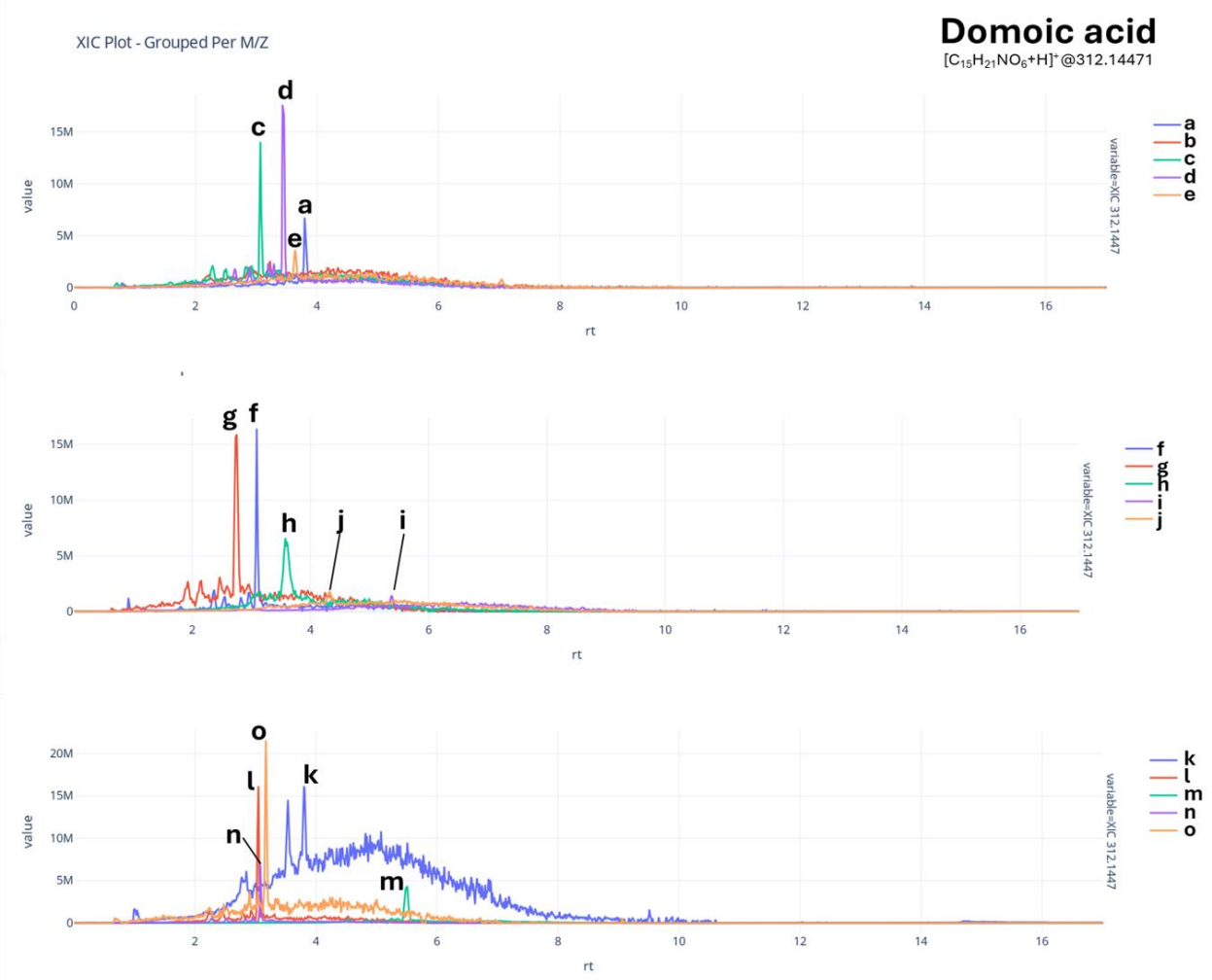

Figure S2: XICs of Domoic acid for the 15 selected datasets.

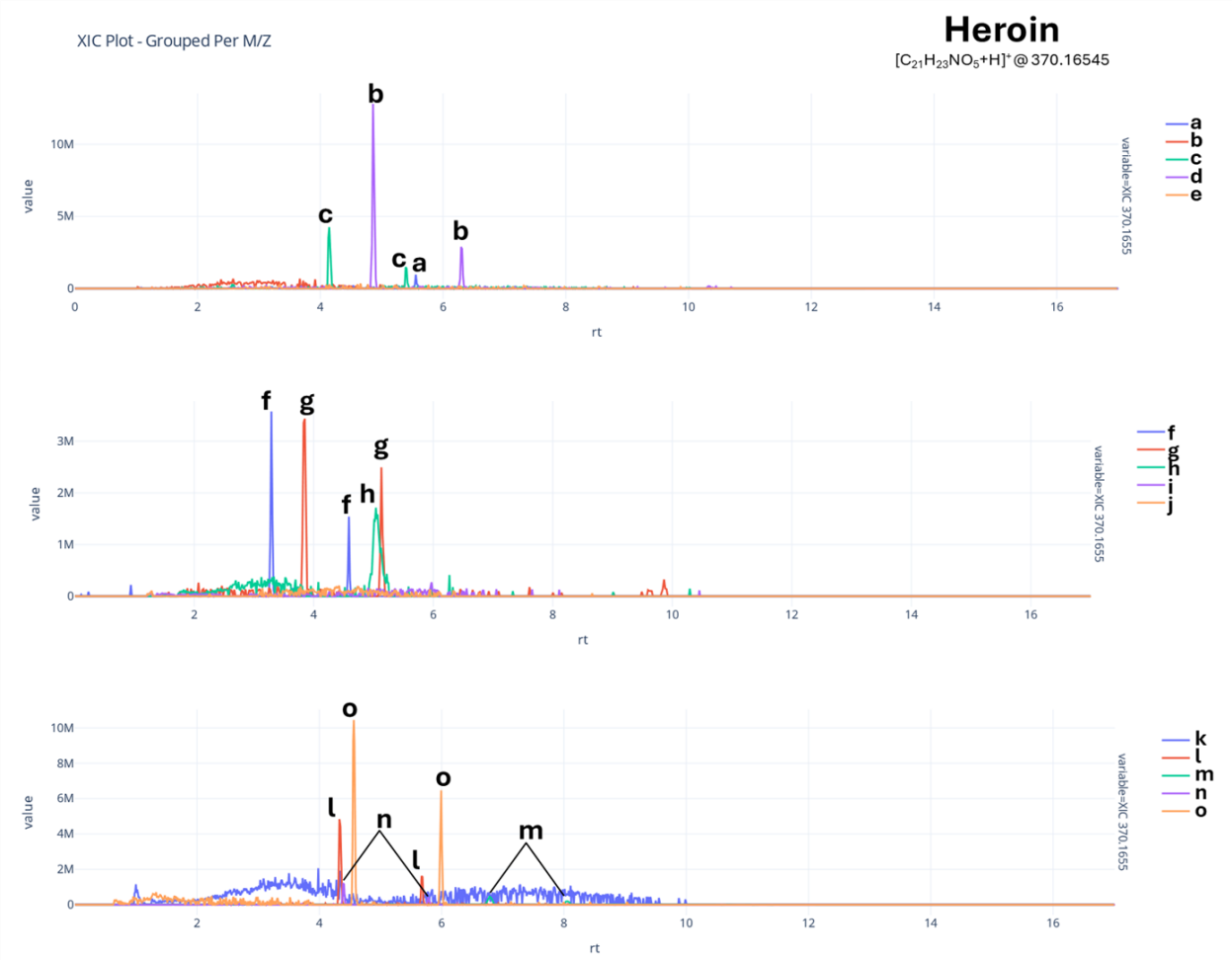

Figure S3: XICs of Heroin for the 15 selected datasets.

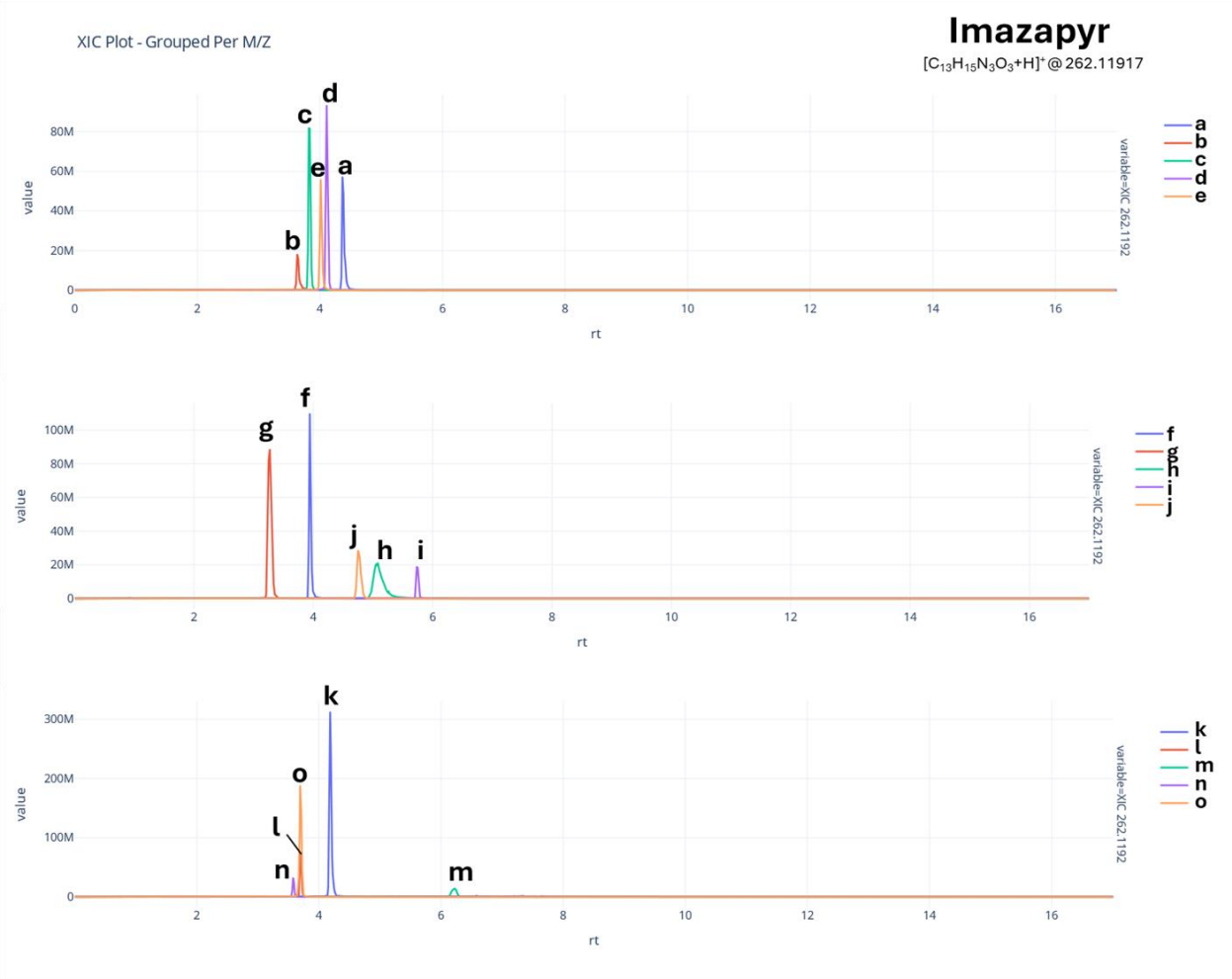

Figure S4: XICs of Imazapyr for the 15 selected datasets.

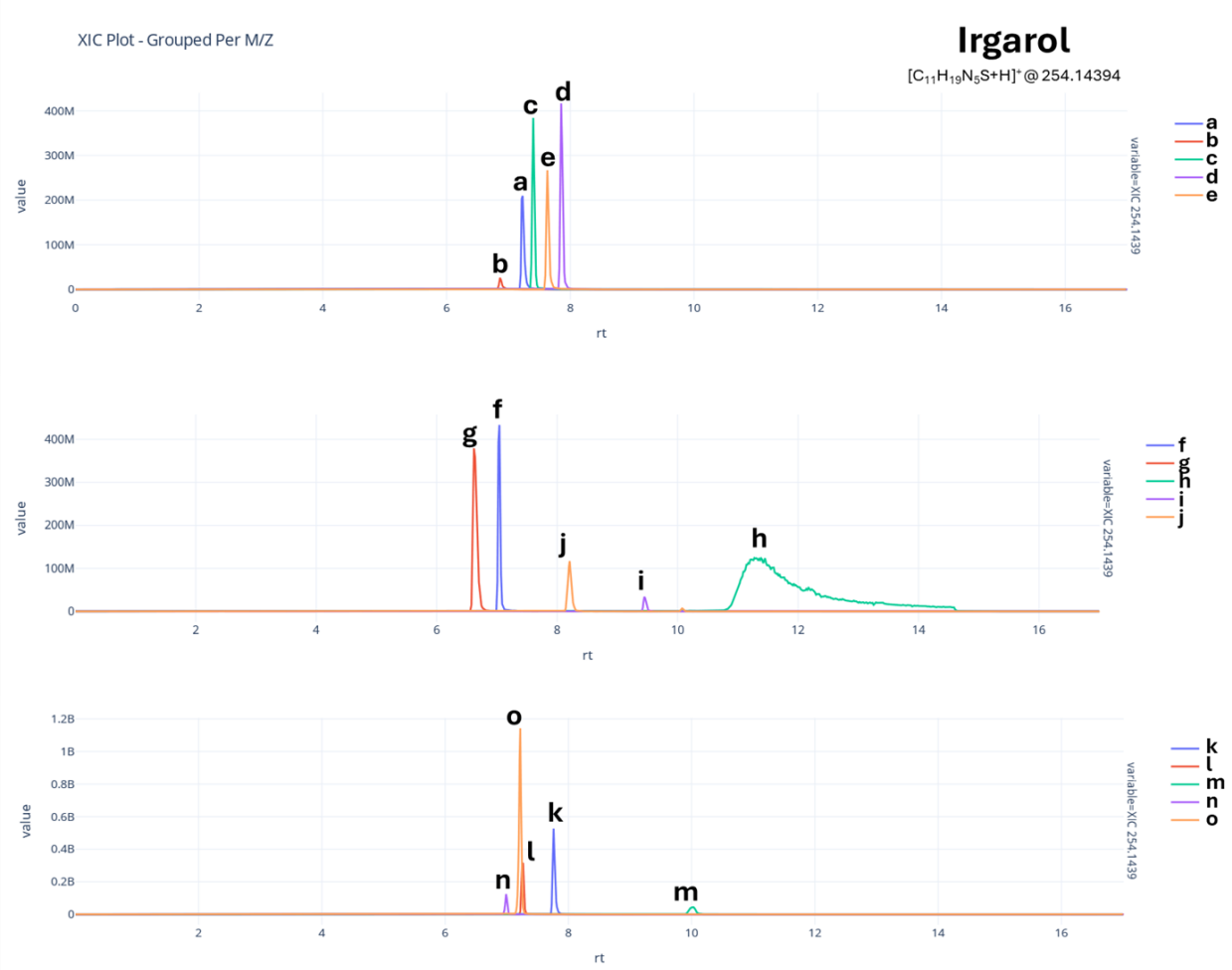

Figure S5: XICs of Irgarol for the 15 selected datasets.

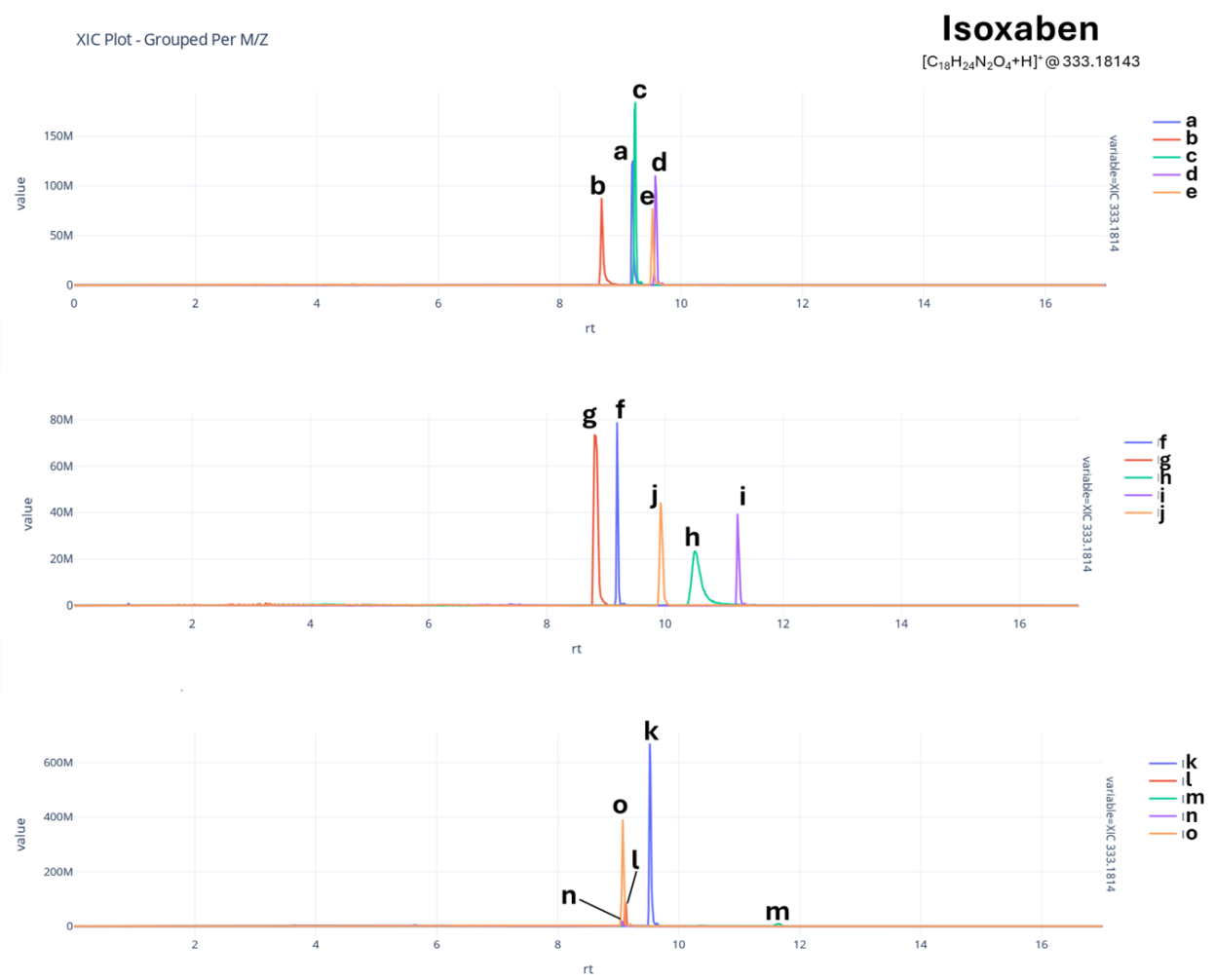

Figure S6: XICs of Isoxaben for the 15 selected datasets.

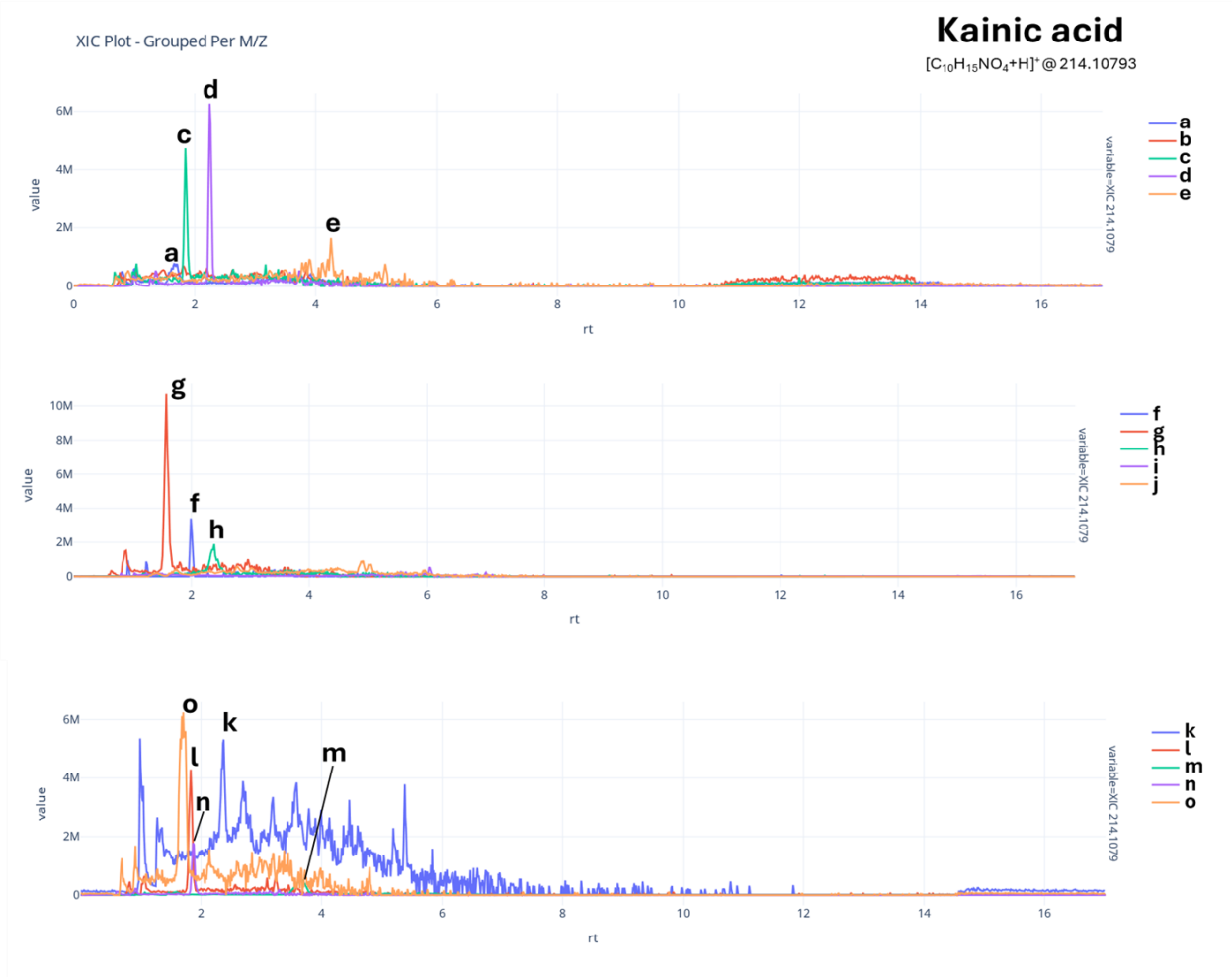

Figure S7: XICs of Kainic acid for the 15 selected datasets.

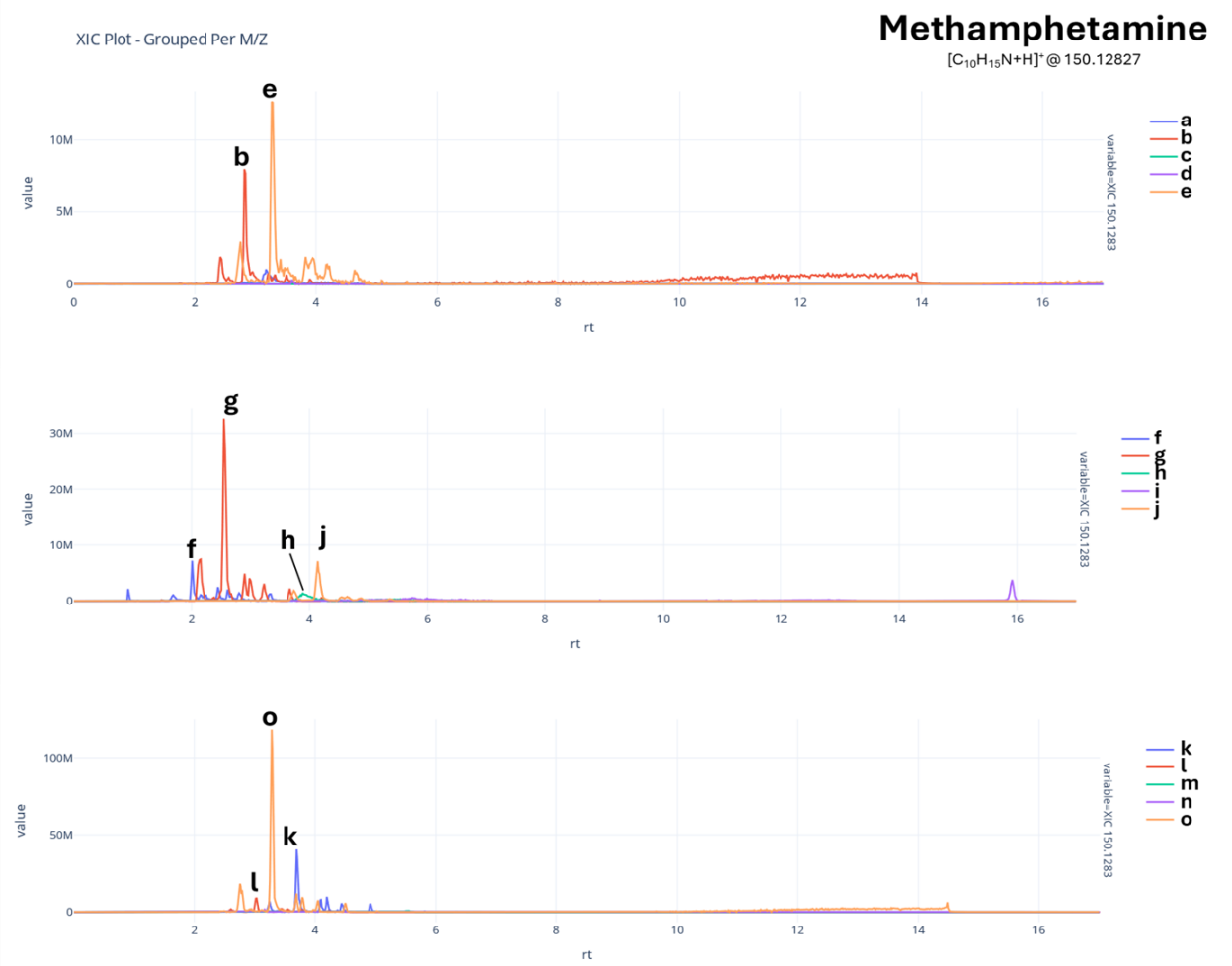

Figure S8: XICs of Methamphetamine for the 15 selected datasets.

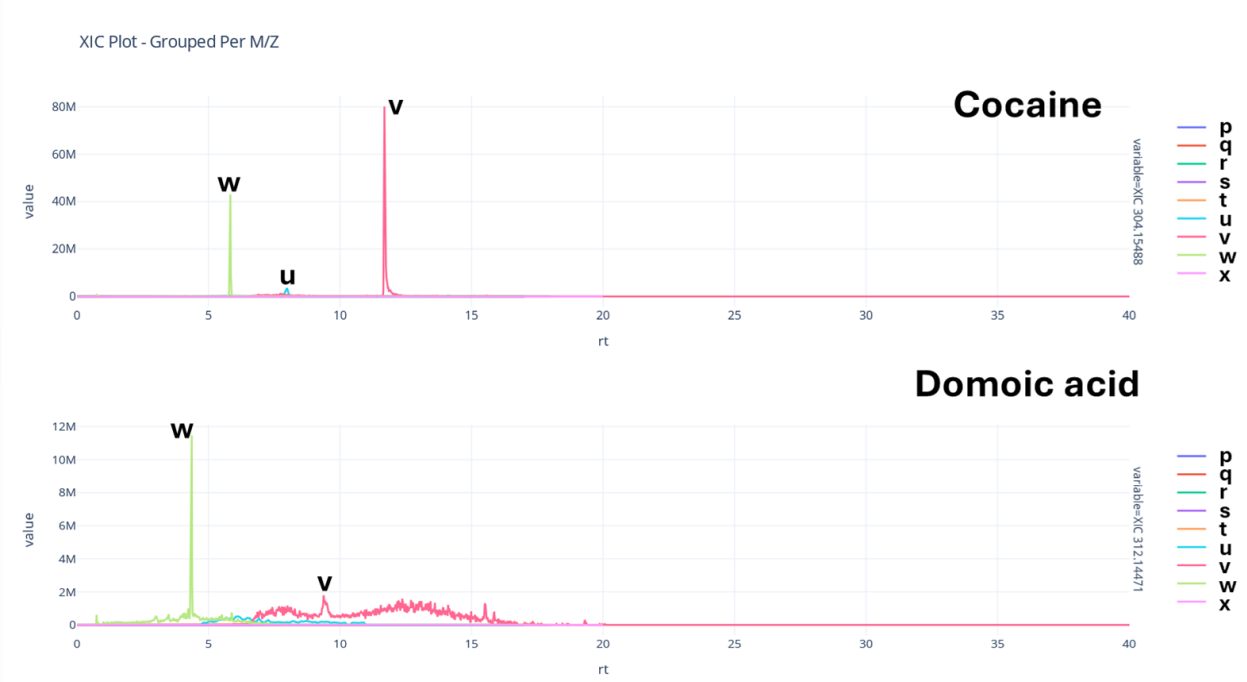

Figure S9: XICs of Cocaine and Domoic acid for the excluded datasets.

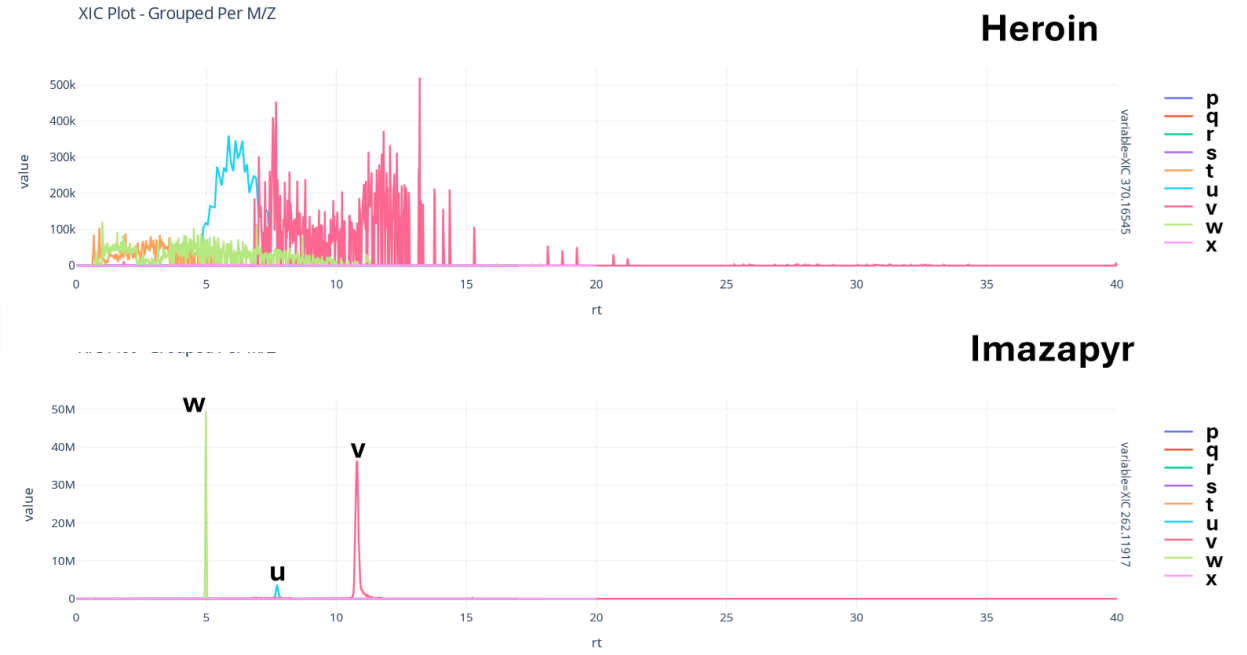

Figure S10: XICs of Heroin and Imazapyr for the excluded datasets.

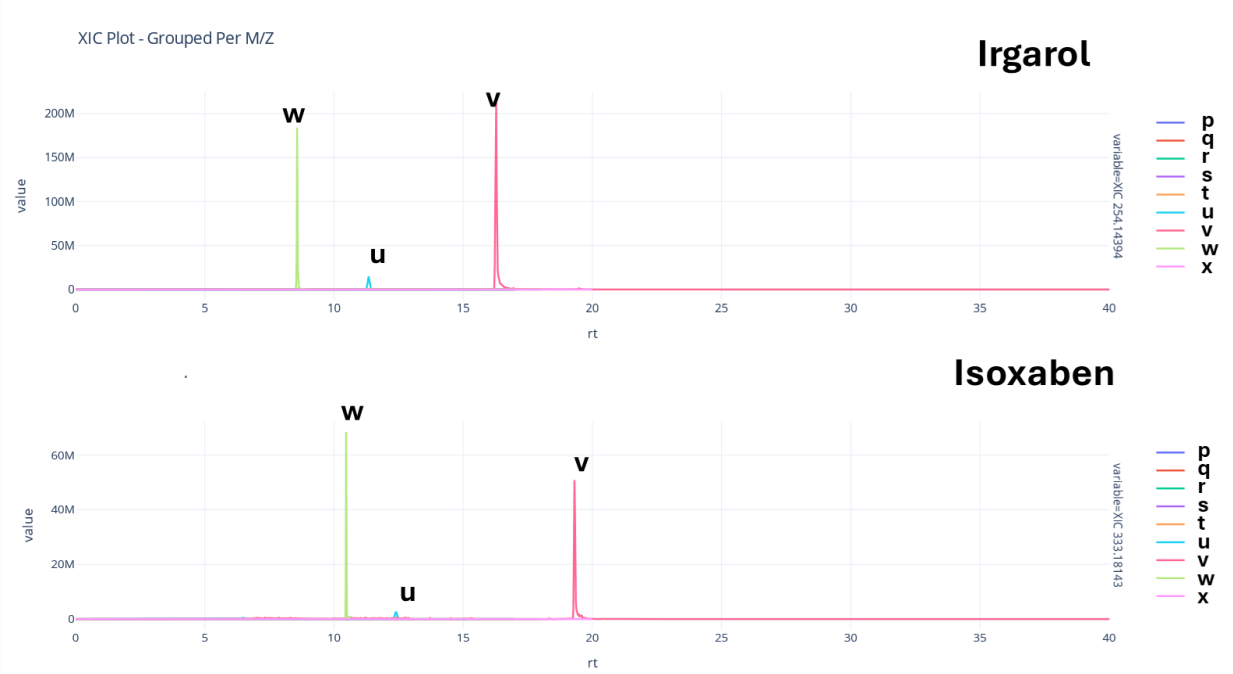

Figure S11: XICs of Irgarol and Isoxaben for the excluded datasets.

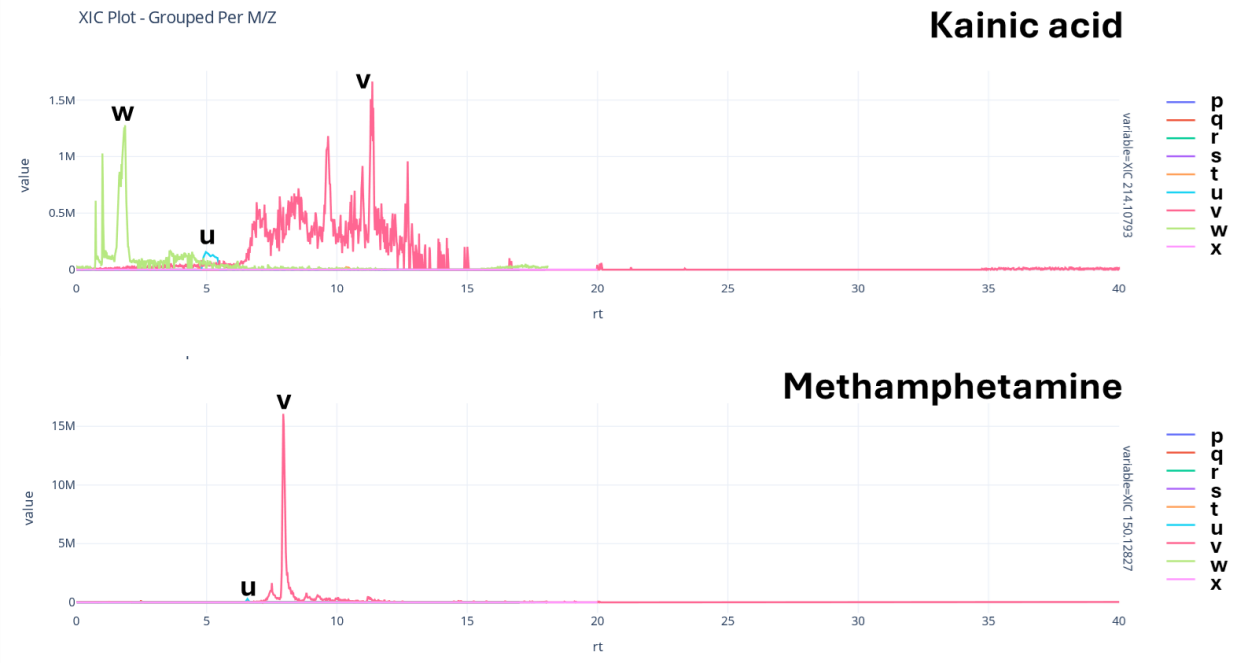

Figure S12: XICs of Kainic acid and Methamphetamine for the excluded datasets.

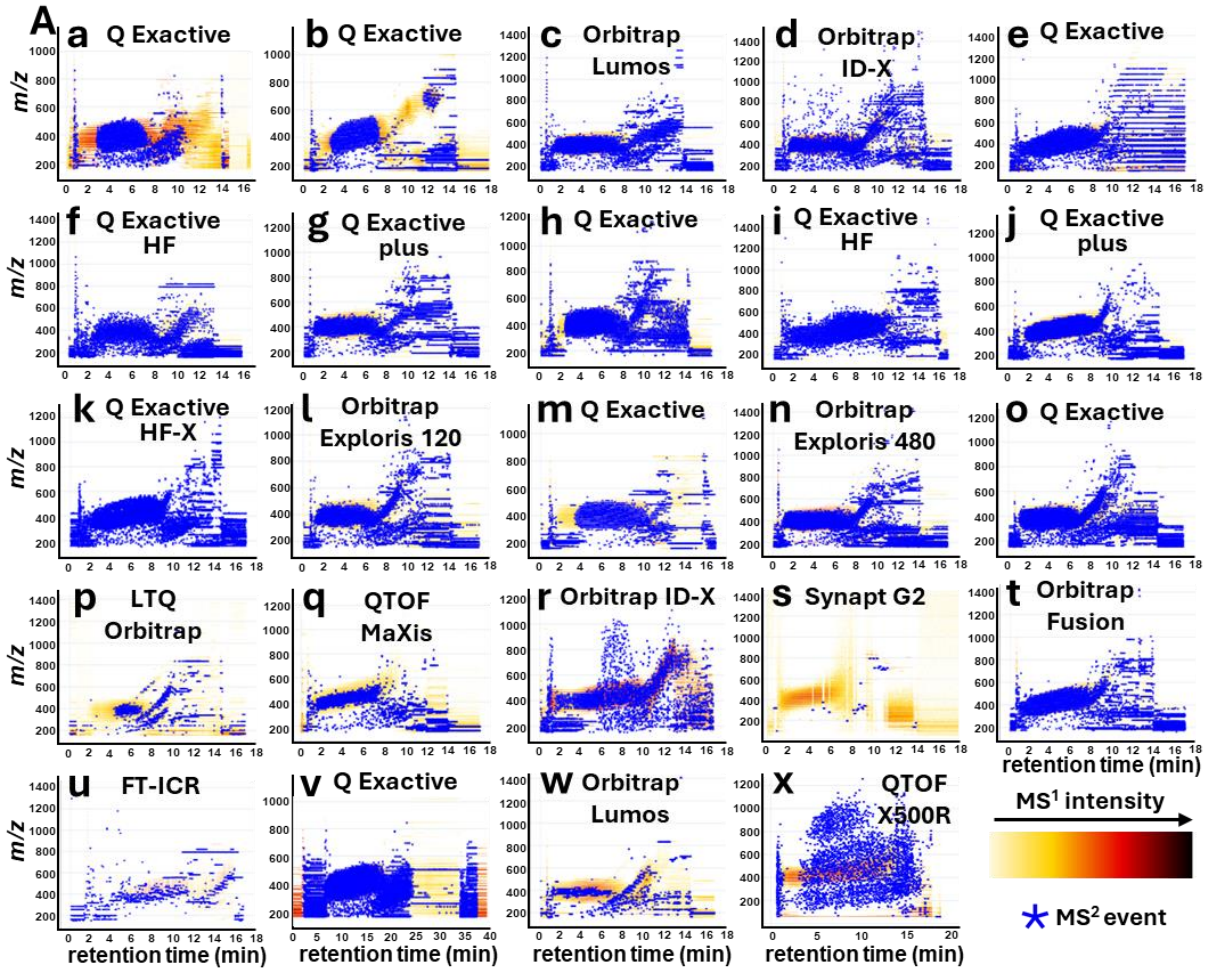

Figure S13: LC-MS/MS (ESI-) heatmaps and MS/MS placement for a representative sample (A45M) for each analyzing laboratory. Datasets from laboratories a–o were selected for subsequent unified analysis.

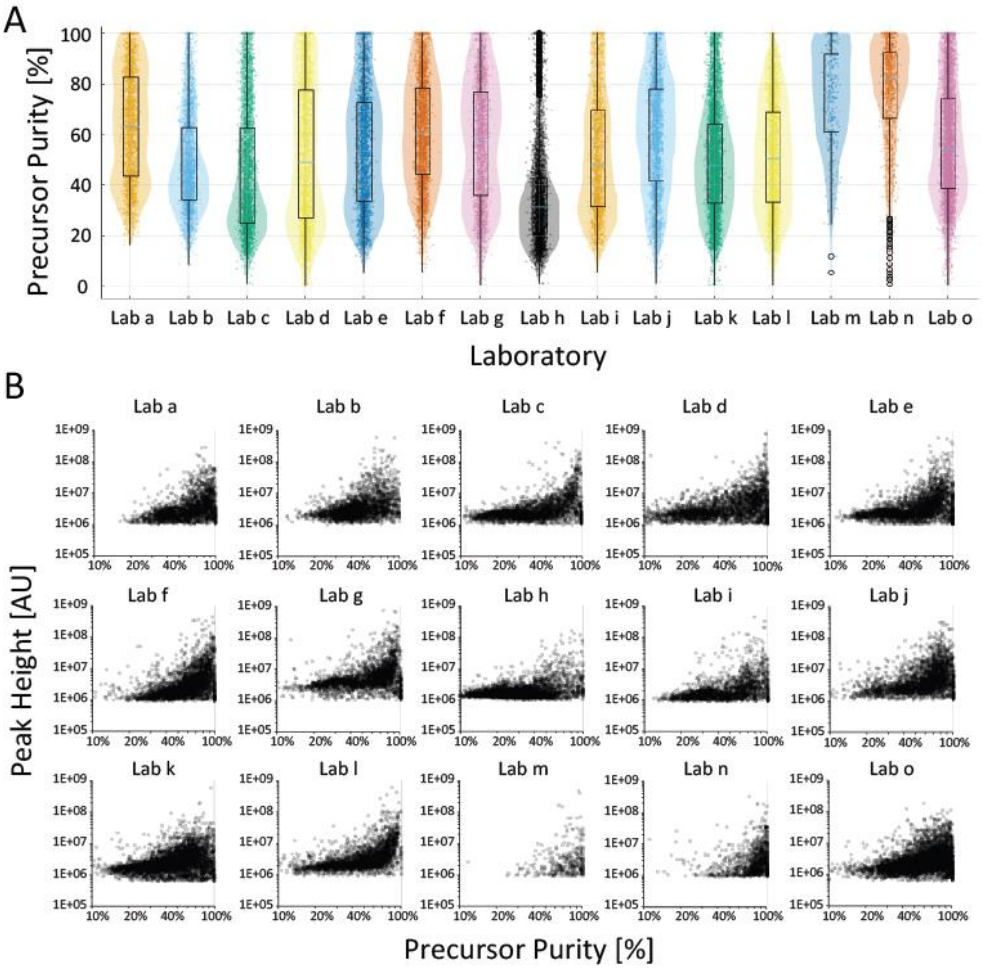

Figure S14: MS/MS precursor purity. (A) shows the raincloud plots of the MS/MS precursor purity between the different data sets. (weighted percentage of the desired precursor in the MS/MS isolation window). The violin shapes depict the full data distribution. The box-and-whisker represents the interquartile range, the horizontal line inside the box marks the median purity, and the whiskers indicate the range of non-outlier values. (B) shows scatter plots that display the relationship between precursor purity and peak height in a double logarithmic scale, between the different datasets.

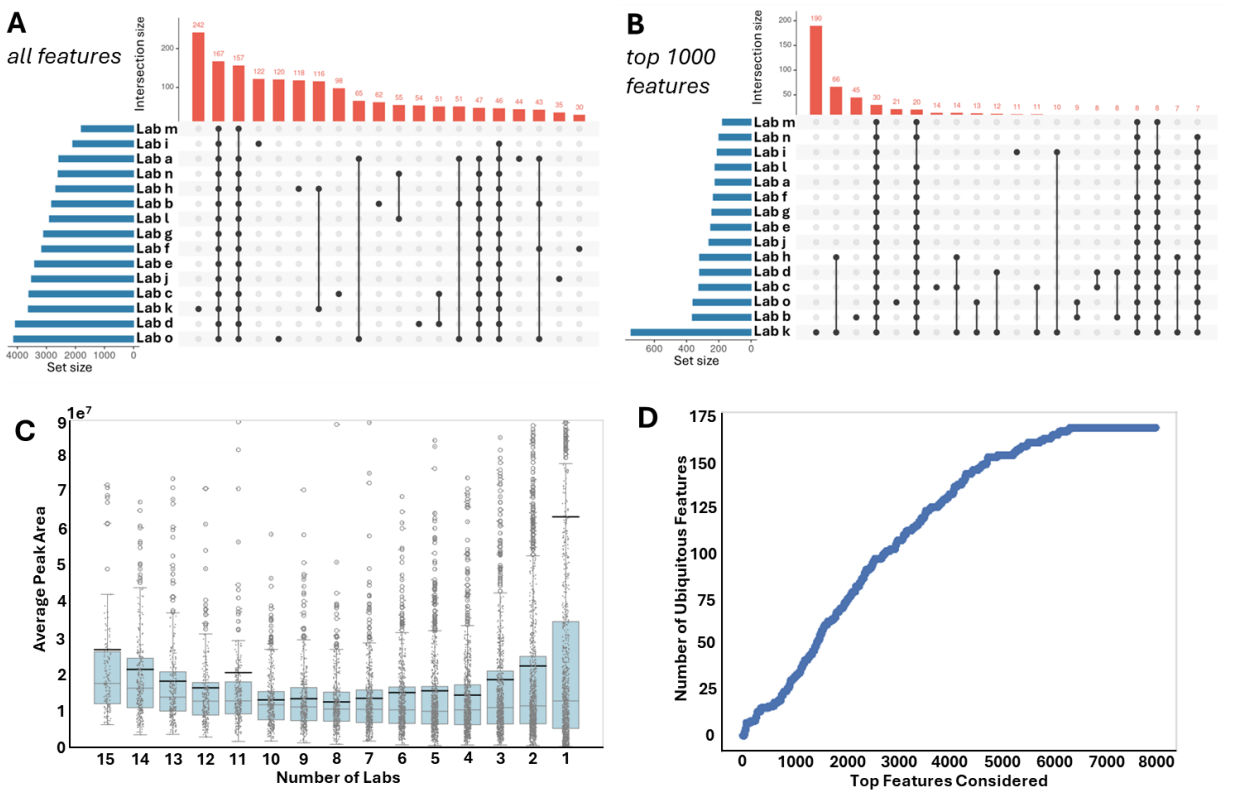

Figure S15: Overview of shared LC-MS/MS features and ubiquity across laboratories. LC-MS/MS (ESI+) data was analyzed with Classical Molecular Networking (CMN). (A) UpSet plot illustrating the distribution of shared features across laboratories when considering all detected features. (B) UpSet plot showing shared feature distributions across laboratories, restricted to the top 1000 most intense features. (C) Boxplot showing average peak area as a function of the number of laboratories in which a feature is observed. (D) Cumulative count of features detected in all laboratories, plotted against feature intensity rank.

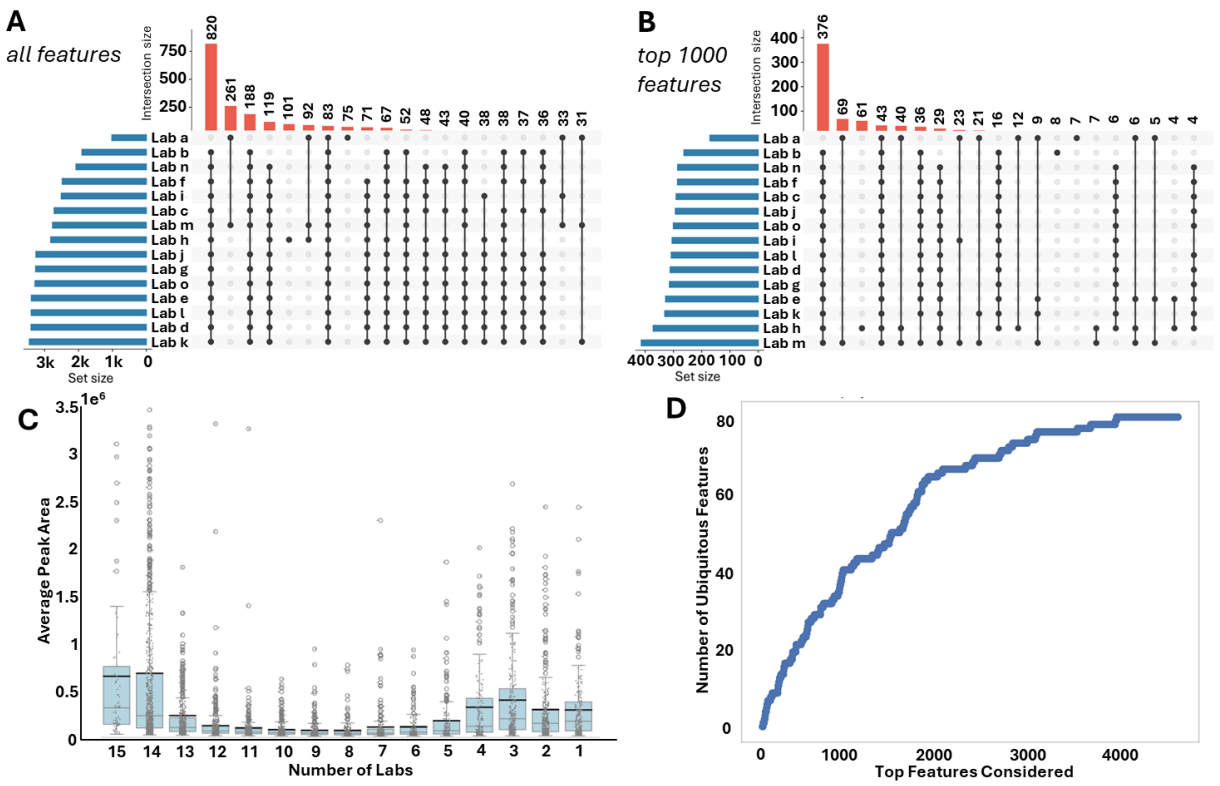

Figure S16: Overview of shared LC-MS/MS features and ubiquity across laboratories. LC-MS/MS (ESI-) data was analyzed with Feature Based Molecular Networking (FBMN). (A) UpSet plot illustrating the distribution of shared features across laboratories when considering all detected features. (B) UpSet plot showing shared feature distributions across laboratories, restricted to the top 1000 most intense features. (C) Boxplot showing average peak area as a function of the number of laboratories in which a feature is observed. (D) Cumulative count of features detected in all laboratories, plotted against feature intensity rank.

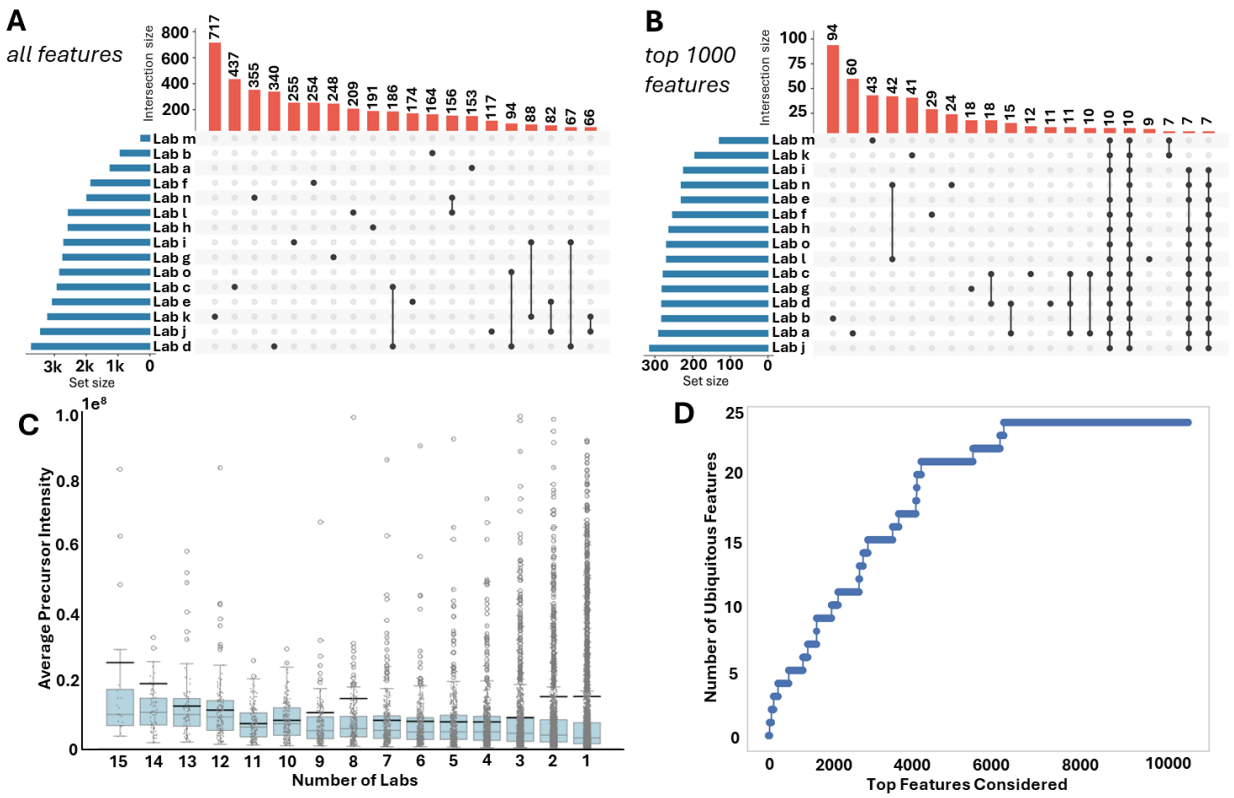

Figure S17: Overview of shared LC-MS/MS features and ubiquity across laboratories. LC-MS/MS (ESI-) data was analyzed with Classical Molecular Networking (CMN). (A) UpSet plot illustrating the distribution of shared features across laboratories when considering all detected features. (B) UpSet plot showing shared feature distributions across laboratories, restricted to the top 1000 most intense features. (C) Boxplot showing average peak area as a function of the number of laboratories in which a feature is observed. (D) Cumulative count of features detected in all laboratories, plotted against feature intensity rank.

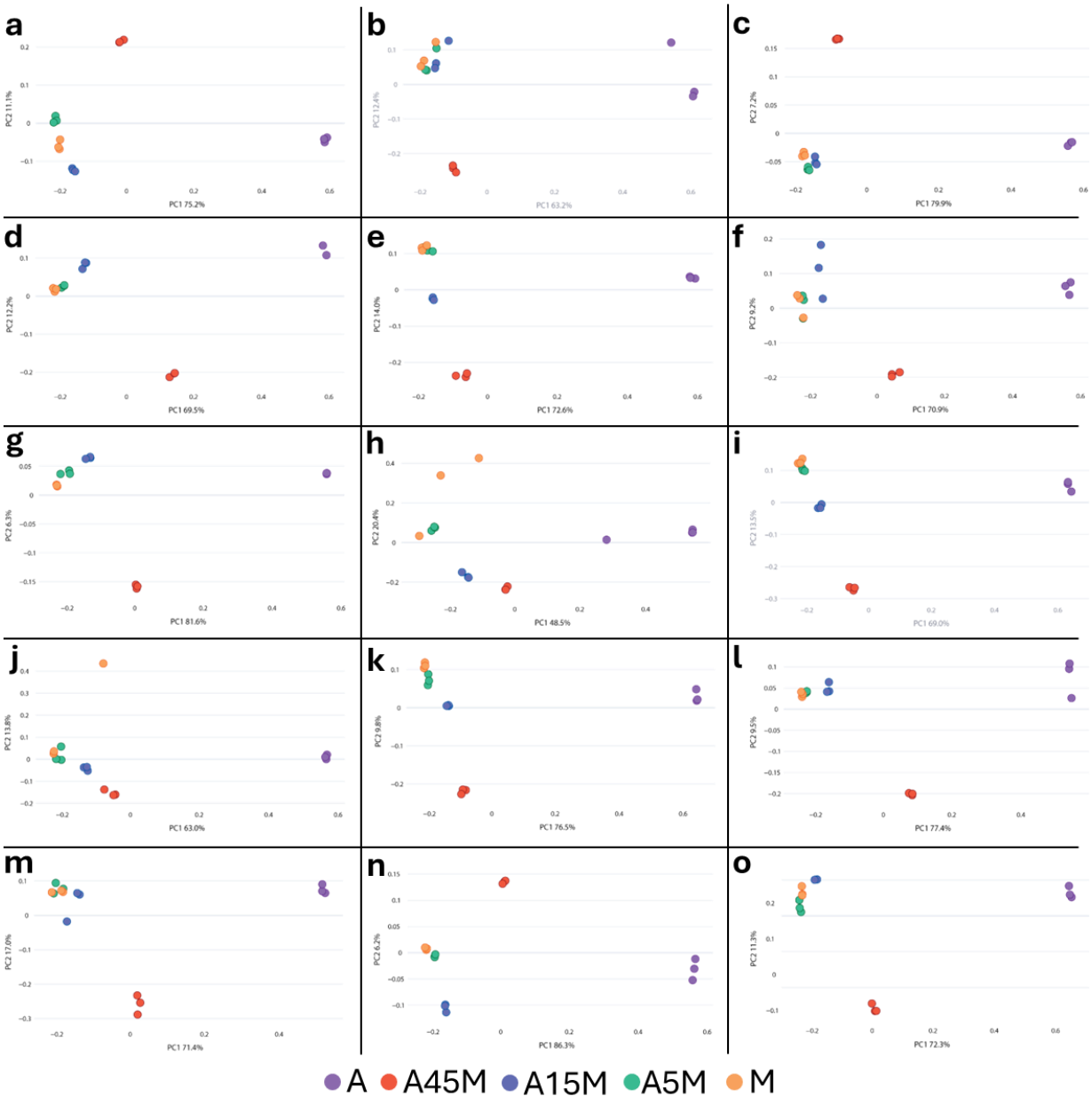

Figure S18: FBMN–PCoAs (ESI+) of individual laboratories, all sample types.

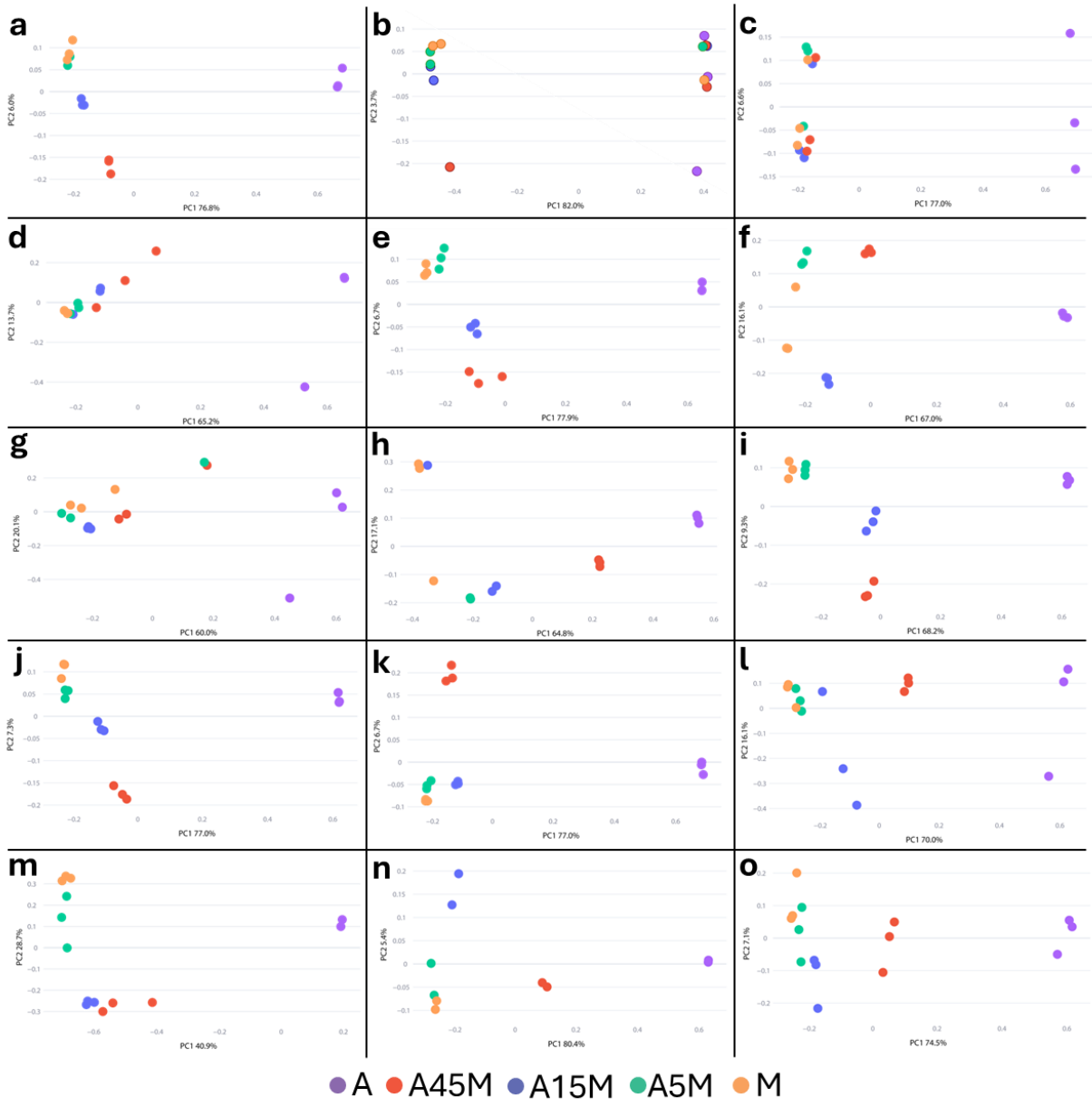

Figure S19: FBMN–PCoAs (ESI-) of individual laboratories, all sample types.

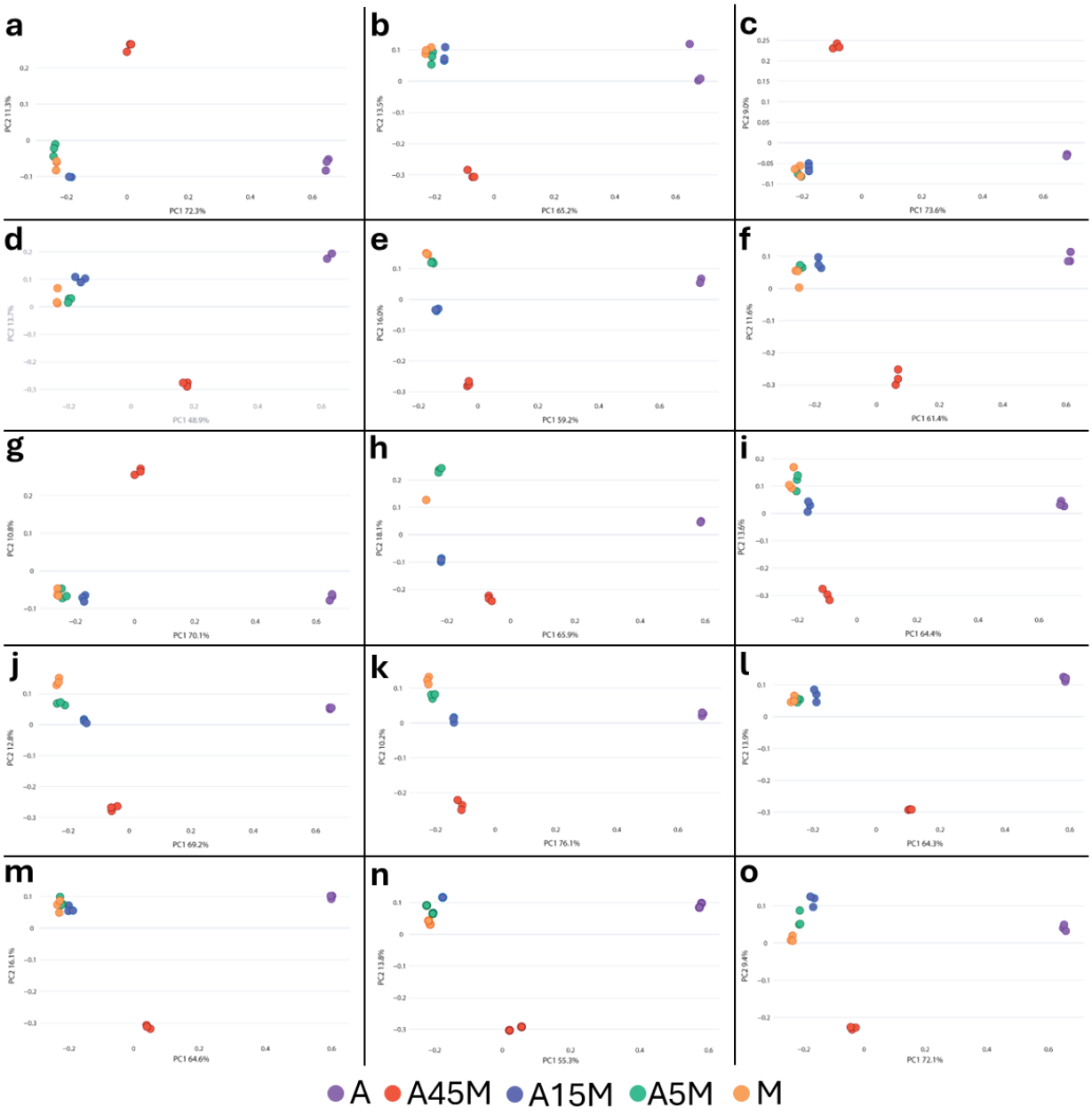

Figure S20: CMN–PCoAs (ESI+) of individual laboratories, all sample types.

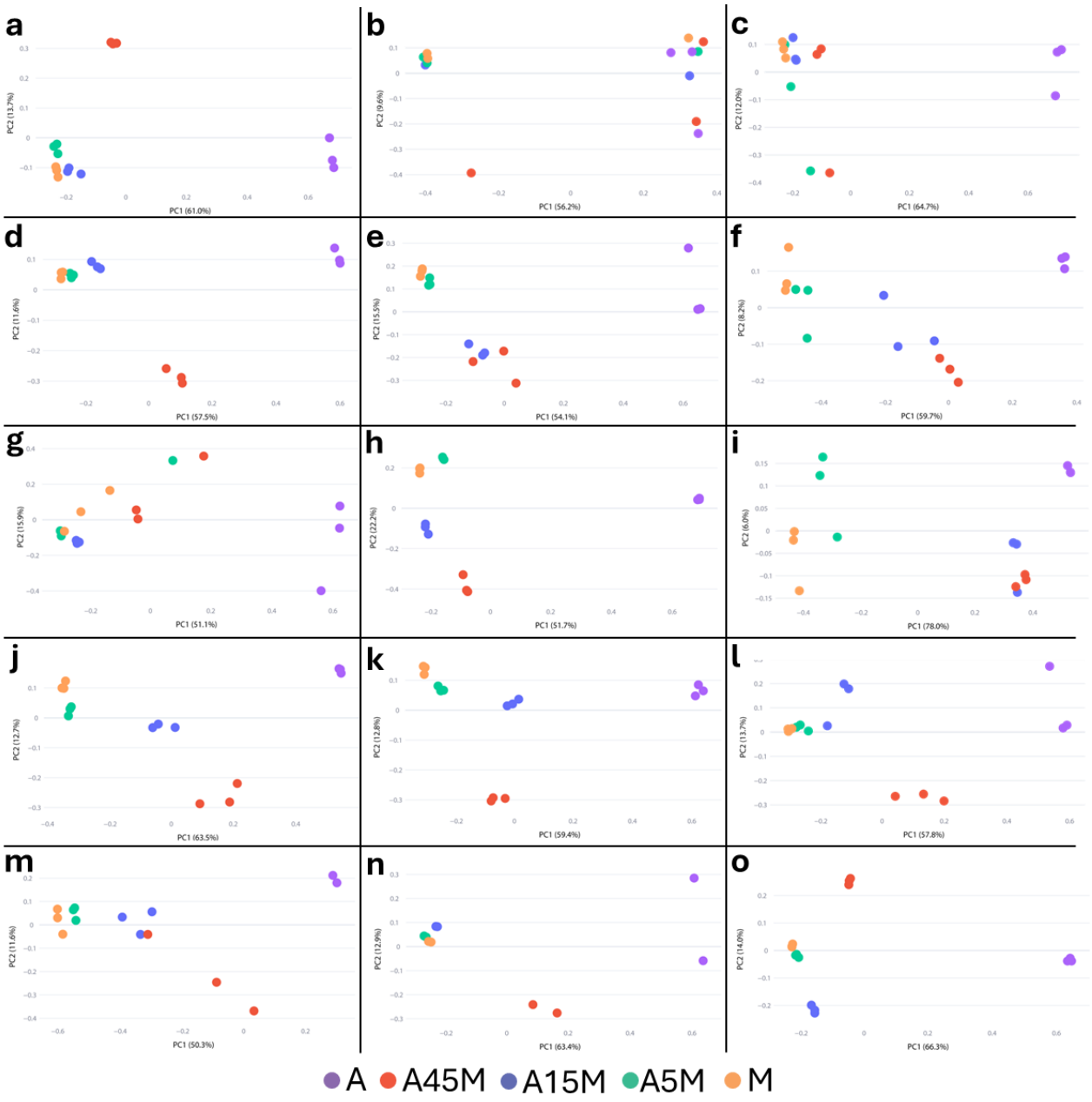

Figure S21: CMN–PCoAs (ESI-) of individual laboratories, all sample types.

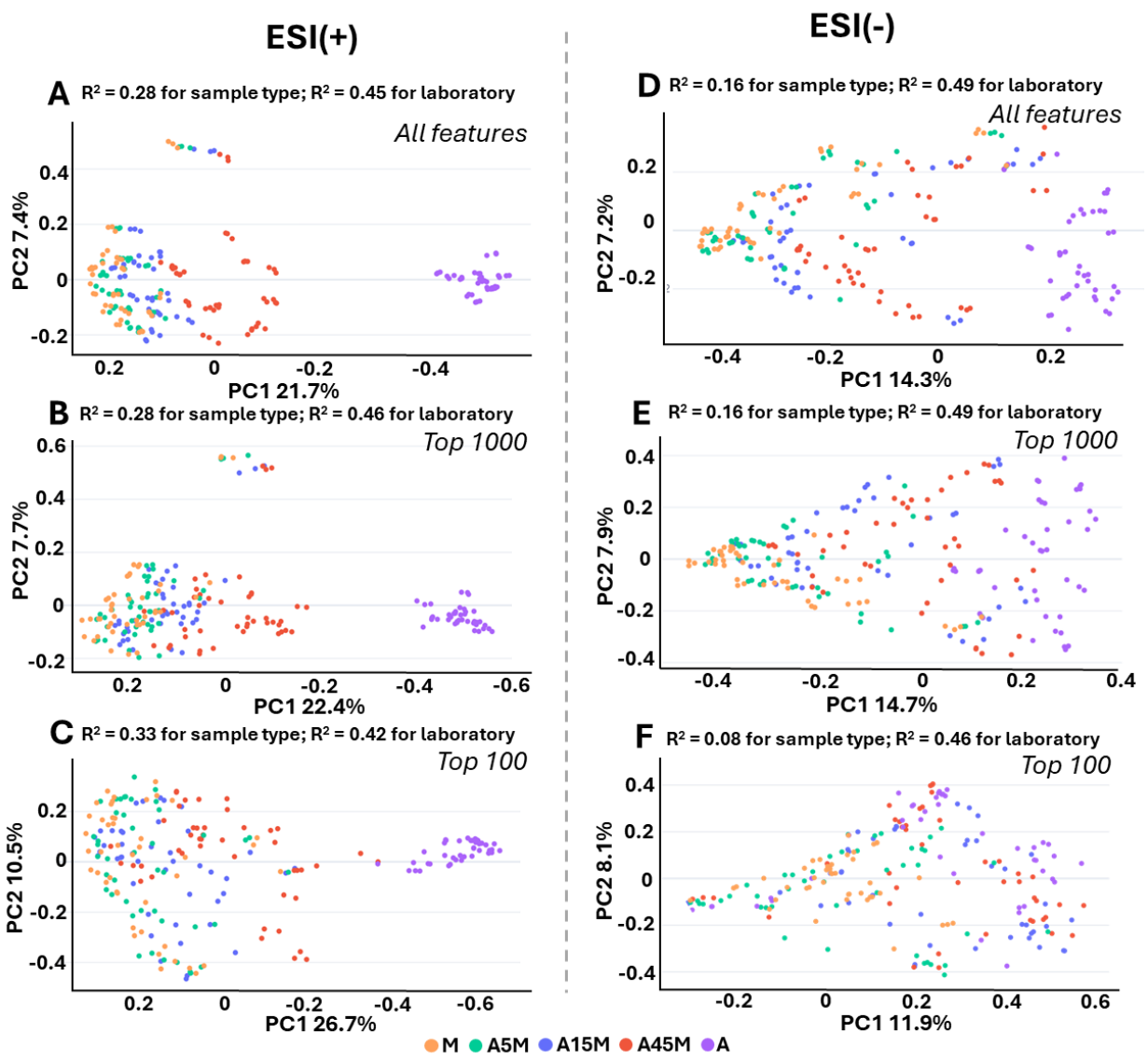

Figure S22: A) PCoA and PERMANOVA using Bray-Curtis dissimilarity for the Classical Molecular Networking analysis (CMN, ESI+). B) for the CMN (ESI+) analysis considering only the top 1000 most intense features (by average feature sum); C) for the CMN (ESI+) analysis considering the top 100 features. D) PCoA and PERMANOVA using Bray-Curtis dissimilarity for the Classical Molecular Networking analysis (CMN, ESI-). E) for the CMN (ESI-) analysis considering only the top 1000 most intense features (by average feature sum); F) for the CMN (ESI-) analysis considering the top 100 features. The colors indicate the different extracts. The colors indicate the different extracts.

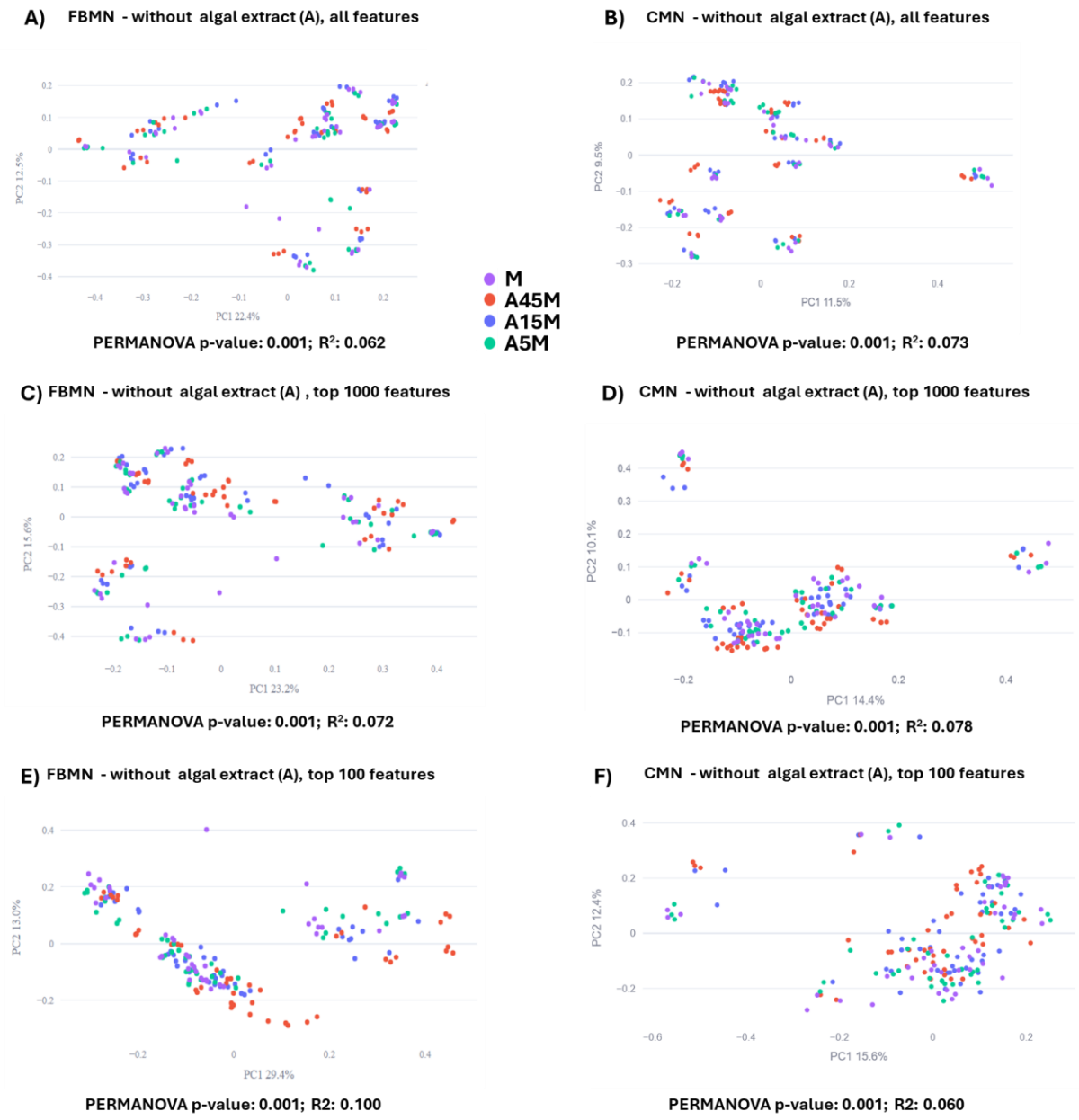

Figure S23: A) PCoA and PERMANOVA for the ESI+ Feature-Based Molecular Networking analysis (FBMN) without samples A. A) all features ; B) PCoA and PERMANOVA for Classical Molecular Networking (CMN) without samples A, all features; C) PCoA and PERMANOVA for the FBMN analysis considering the top 1000 features, without samples A; D) PCoA and PERMANOVA for the CMN analysis considering the top 1000 features without samples A; E) PCoA and PERMANOVA for the FBMN analysis considering the top 100 features, without samples A; F) PCoA and PERMANOVA for the CMN analysis considering the top 100 features, without samples A.

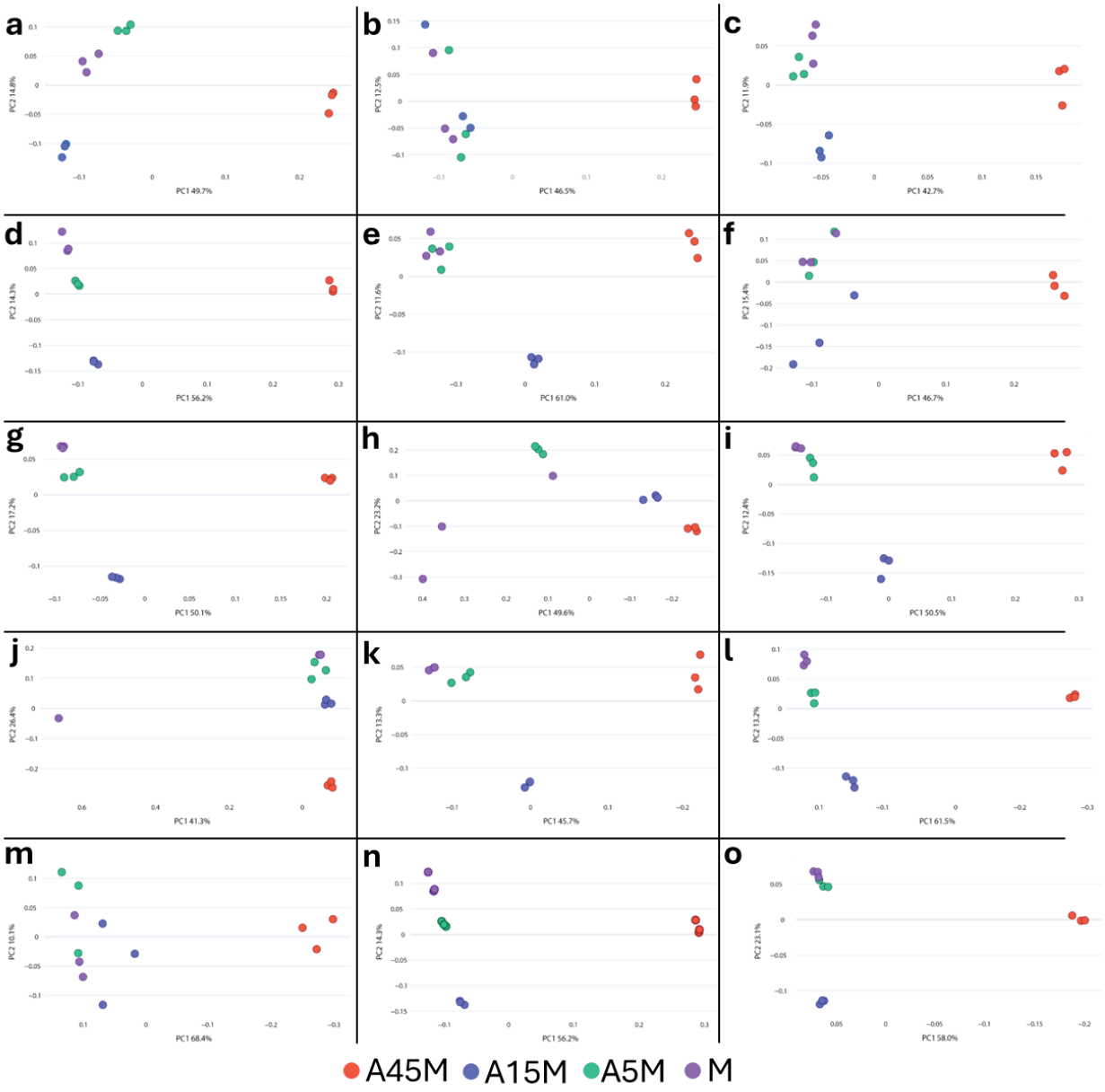

Figure S24: FBMN–PCoAs of individual laboratories, without samples A (ESI+).

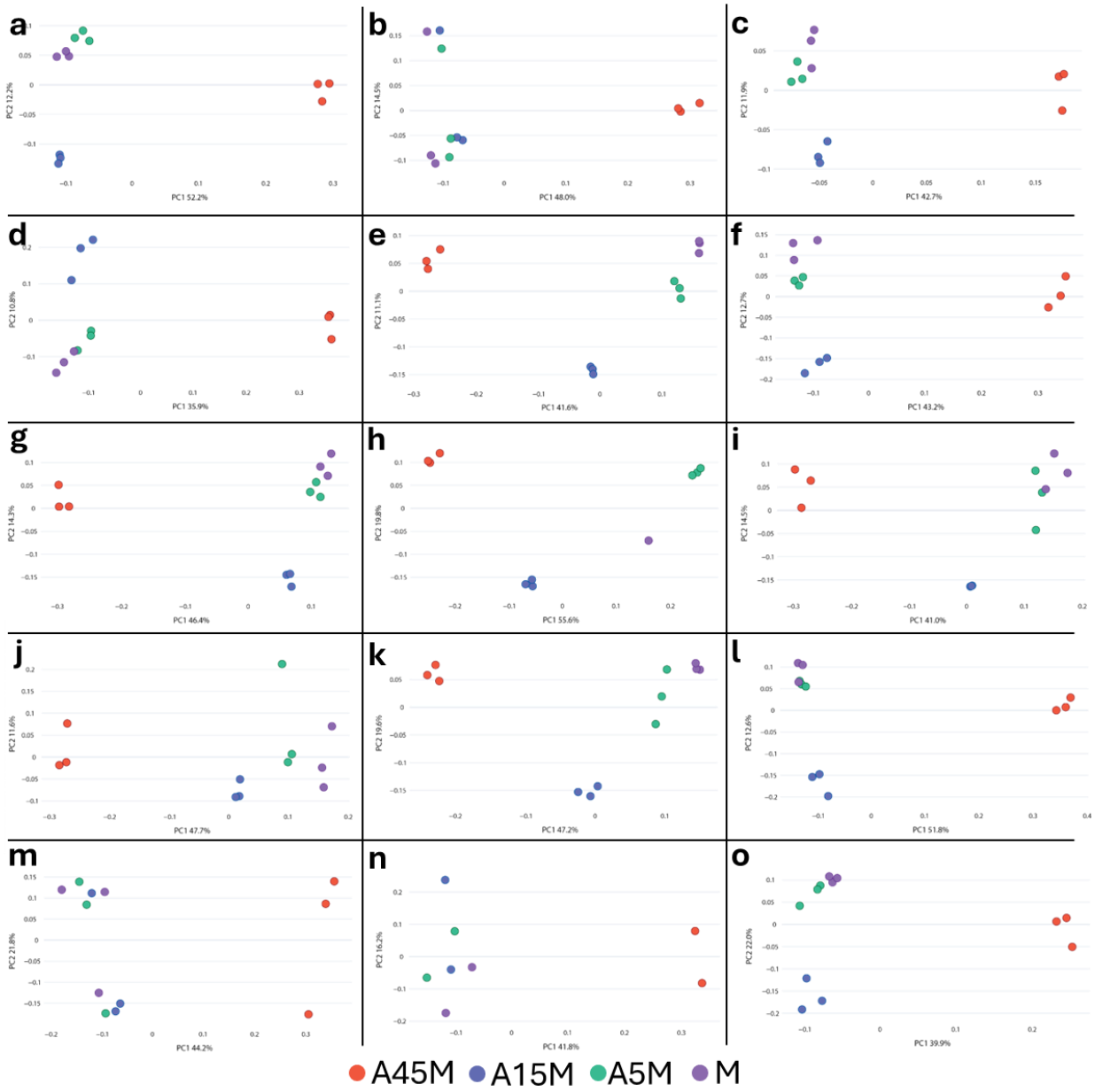

Figure S25: CMN–PCoAs of individual laboratories, without samples A (ESI+).

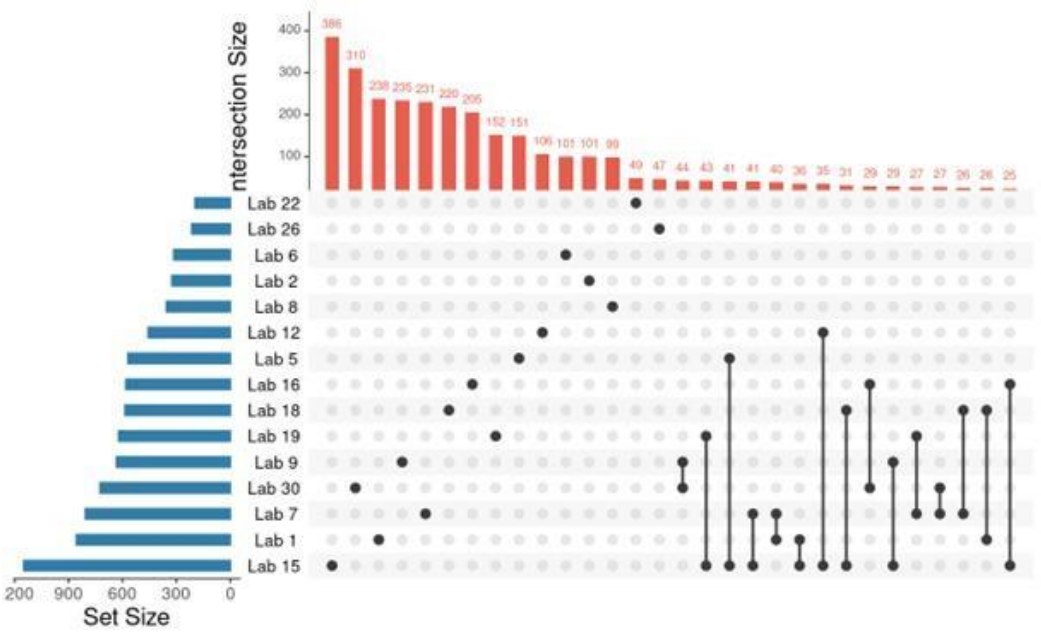

Figure S26: Random Forest (RF) analysis results of ESI+ features driving sample type clustering depicted as an UpSet plot showing the overlap of significant features identified by RF analysis across laboratories using feature-based molecular networking (FBMN) without merging of features based on identical spectral library annotation.

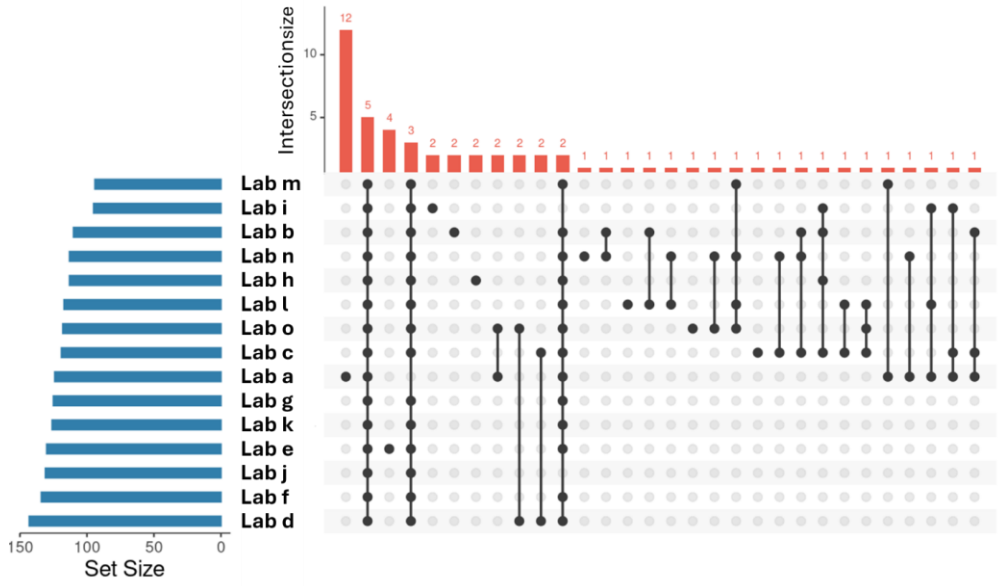

Figure S27: Random Forest (RF) analysis of ESI+ features driving sample type clustering. UpSet plot showing the overlap of significant features identified by RF analysis across laboratories using classical molecular networking (CMN) followed by merging of features based on identical spectral library annotation.