Supporting Information: Ion mobility, hydrogen/deuterium exchange, and isotope scrambling: Tools to aid compound identification in 'omics mixtures

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Table S1. Drift times for assigned peptide ions from experimental runs employing random mobility selection.

Peptide Ion[a]	*m/z*[b]	Run 1[c]	Run 2[c]	Run 3[c]	Average[d]	CV[e]
$[QRLIFAGKQ+3H]^{3+}$	354.58	6.96	7.01	6.93	6.97	0.58
$[NIQ+H]^{+}$	374.27	8.25	8.30	8.22	8.26	0.49
$[HLVLRL+2H]^{2+}$	375.77	6.91	6.97	6.90	6.93	0.55
$[GIPPDQQ+2H]^{2+}$	377.75	6.33	6.39	6.35	6.36	0.48
$[KIQDKE+2H]^{2+}$	380.72	6.20	6.25	6.20	6.22	0.46
$[VLRLRGG+2H]^{2+}$	385.77	6.94	6.98	6.92	6.95	0.44
$[IFAGKQL+2H]^{2+}$	388.75	6.07	6.11	6.10	6.09	0.34
$[DKE+H]^{+}$	391.24	8.46	8.55	8.43	8.48	0.74
$[VKTLTGKTITL+3H]^{3+}$	392.28	7.11	7.07	7.05	7.08	0.43
$[NIQKESTLHL+3H]^{3+}$	394.92	6.46	6.50	6.46	6.47	0.36
$[KESTLHL+2H]^{2+}$	414.25	6.49	6.56	6.52	6.52	0.54
$[QRL+H]^{+}$	416.26	8.51	8.56	8.47	8.51	0.53
$[NVKAA]^{+}$	431.33	9.07	9.16	9.08	9.10	0.54
$[NIQKESTL+2H]^{2+}$	466.79	7.61	7.70	7.60	7.64	0.72
$[HLVL]^{+}$	481.34	9.42	9.49	9.40	9.44	0.50
$[VLRL]^{+}$	500.40	10.62	10.73	10.60	10.65	0.66

[a]Peptide ion assignments based on m/z matches to monoisotopic peaks and charge (obtained from isotopic distrbution)

[b]Experimental *m/z* values for monoisotopic peaks from the 2D datasets

[c]Ion drift times in ms as obtained from 2D dataset centroids for the assigned ions

[d]Average ion drift times in ms for the three experimental runs

[e]Coefficients of variation for the drift times expressed as a percentage

Table S2. Drift times for assigned peptide ions from experimental runs employing sequential mobility selection.

Peptide Ion[a]	*m/z*[b]	Run 1[c]	Run 2[c]	Run 3[c]	Average[d]	CV[e]
$[QRLIFAGKQ+3H]^{3+}$	354.58	7.00	6.96	6.99	6.98	0.30
$[NIQ+H]^{+}$	374.27	8.26	8.23	8.20	8.22	0.26
$[HLVLRL+2H]^{2+}$	375.77	6.93	6.91	6.91	6.91	0.00
$[GIPPDQQ+2H]^{2+}$	377.75	6.31	6.35	6.29	6.32	0.67
$[KIQDKE+2H]^{2+}$	380.72	6.23	6.20	6.18	6.19	0.23
$[VLRLRGG+2H]^{2+}$	385.77	6.98	6.93	6.91	6.92	0.20
$[IFAGKQL+2H]^{2+}$	388.75	6.07	6.00	6.06	6.03	0.70
$[DKE+H]^{+}$	391.24	8.47	8.40	8.50	8.45	0.84
$[VKTLTGKTITL+3H]^{3+}$	392.28	7.08	7.07	7.08	7.08	0.10
$[NIQKESTLHL+3H]^{3+}$	394.92	6.50	6.48	6.46	6.47	0.22
$[KESTLHL+2H]^{2+}$	414.25	6.51	6.50	6.47	6.49	0.33
$[QRL+H]^{+}$	416.26	8.53	8.46	8.51	8.49	0.42
$[NVKAA]^{+}$	431.33	9.08	9.03	9.08	9.06	0.39
$[NIQKESTL+2H]^{2+}$	466.79	7.68	7.65	7.67	7.66	0.18
$[HLVL]^{+}$	481.34	9.44	9.40	9.43	9.42	0.23
$[VLRL]^{+}$	500.40	10.68	10.70	10.66	10.68	0.26

[a]Peptide ion assignments based on m/z matches to monoisotopic peaks and charge (obtained from isotopic distrbution)

[b]Experimental *m/z* values for monoisotopic peaks from the 2D datasets

[c]Ion drift times in ms as obtained from 2D dataset centroids for the assigned ions

[d]Average ion drift times in ms for the three experimental runs

[e]Coefficients of variation for the drift times expressed as a percentage

Table S3. Drift times for D_2O-exposed peptide ions from experimental runs employing sequential mobility selection.

Peptide Ion[a]	*m/z*[b]	Run 1[c]	Run 2[c]	Run 3[c]	Average[d]	CV[e]
$[QRLIFAGKQ+3H]^{3+}$	354.58	8.77	8.65	8.65	8.69	0.80
$[NIQ+H]^{+}$	374.27	10.36	10.30	10.33	10.33	0.29
$[HLVLRL+2H]^{2+}$	375.77	8.99	8.89	8.92	8.93	0.57
$[GIPPDQQ+2H]^{2+}$	377.75	8.51	8.40	8.39	8.43	0.79
$[KIQDKE+2H]^{2+}$	380.72	8.32	8.23	8.20	8.25	0.76
$[VLRLRGG+2H]^{2+}$	385.77	9.02	8.92	9.00	8.98	0.59
$[IFAGKQL+2H]^{2+}$	388.75	7.80	7.74	7.80	7.78	0.45
$[DKE+H]^{+}$	391.24	10.42	10.36	10.42	10.40	0.33
$[VKTLTGKTITL+3H]^{3+}$	392.28	8.96	8.90	8.95	8.94	0.36
$[NIQKESTLHL+3H]^{3+}$	394.92	8.48	8.40	8.40	8.43	0.55
$[KESTLHL+2H]^{2+}$	414.25	8.60	8.52	8.50	8.54	0.62
$[QRL+H]^{+}$	416.26	10.47	10.40	10.42	10.43	0.35
$[NVKAA]^{+}$	431.33	10.96	10.82	10.90	10.89	0.64
$[NIQKESTL+2H]^{2+}$	466.79	9.47	9.60	9.45	9.51	0.86
$[HLVL]^{+}$	481.34	11.40	11.30	11.43	11.38	0.60
$[VLRL]^{+}$	500.40	12.33	12.42	12.56	12.44	0.93

[a]Peptide ion assignments based on m/z matches to monoisotopic peaks and charge (obtained from isotopic distrbution)

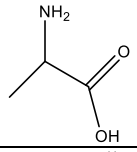
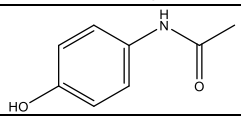
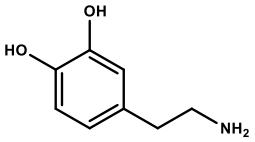
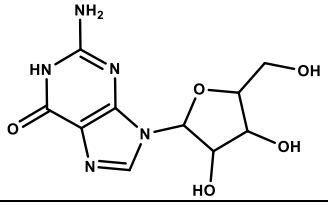
[b]Experimental *m/z* values for monoisotopic peaks from the 2D datasets

[c]Ion drift times in ms as obtained from 2D dataset centroids for the assigned ions

[d]Average ion drift times in ms for the three experimental runs

[e]Coefficients of variation for the drift times expressed as a percentage

Table S4. Structures of metabolite compounds used for the IMS-HDX-MS experiments and their reduced mobilities.

Name	Nominal *m/z*[a]	Structure[b]	Reduced Mobility[c]
Alanine	90		8.8×10^{-4}
Acetaminophen	152		7.8×10^{-4}
Dopamine	154		8.0×10^{-4}
Guanosine	284		5.8×10^{-4}

[a] Nominal *m/z* associated with the monoisotopic peak for the respective compound
[b] Molecular structure obtained from ChemDraw freeware
[c] Reduced mobilities ($m^2 \cdot V^{-1} \cdot s^{-1}$) recorded for the respective compounds from measurements using He buffer gas.

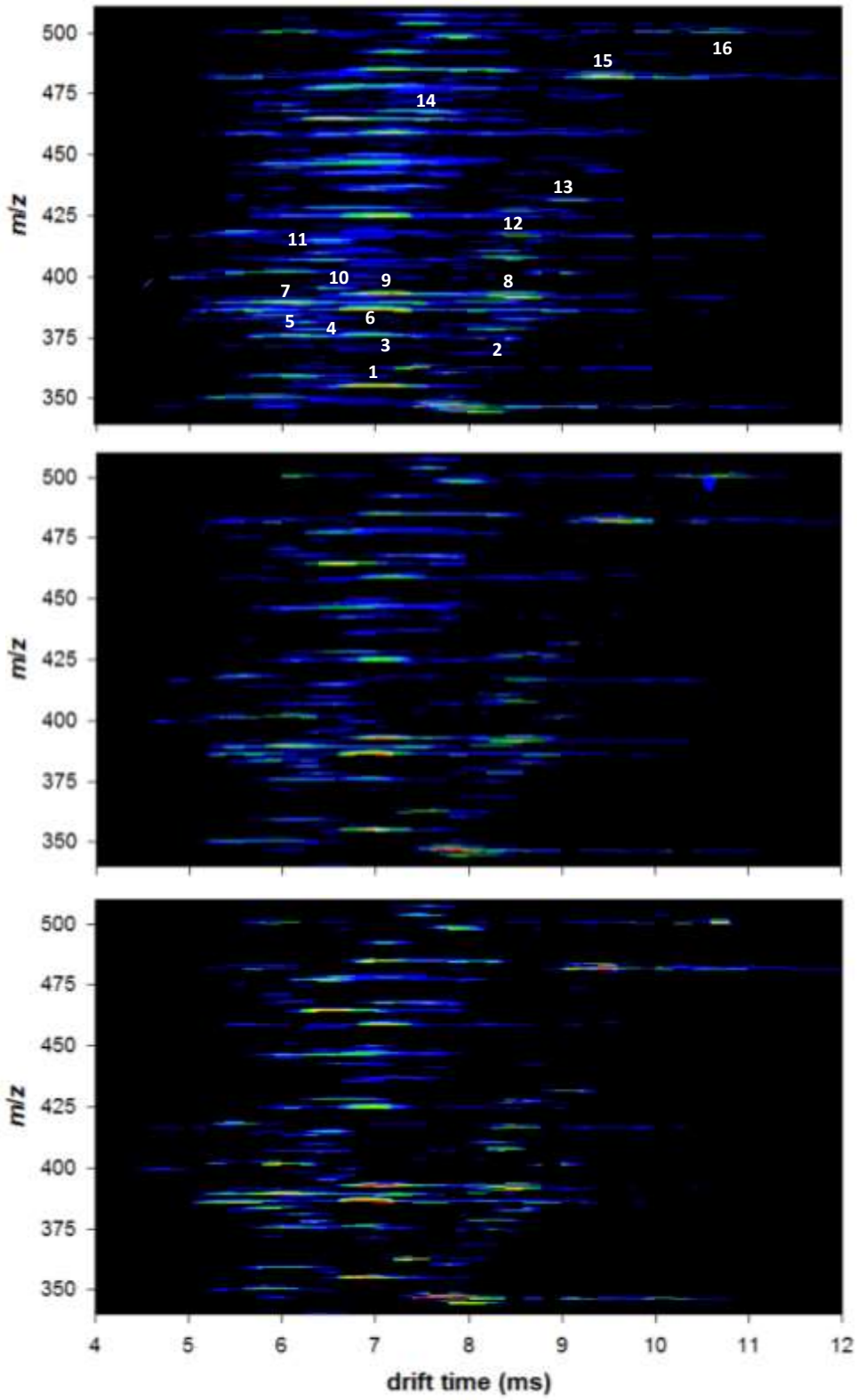

500
475
450
425
400
375
350
m/z
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
4
5
6
7
8
9
10
11
12
drift time (ms)

Figure S1

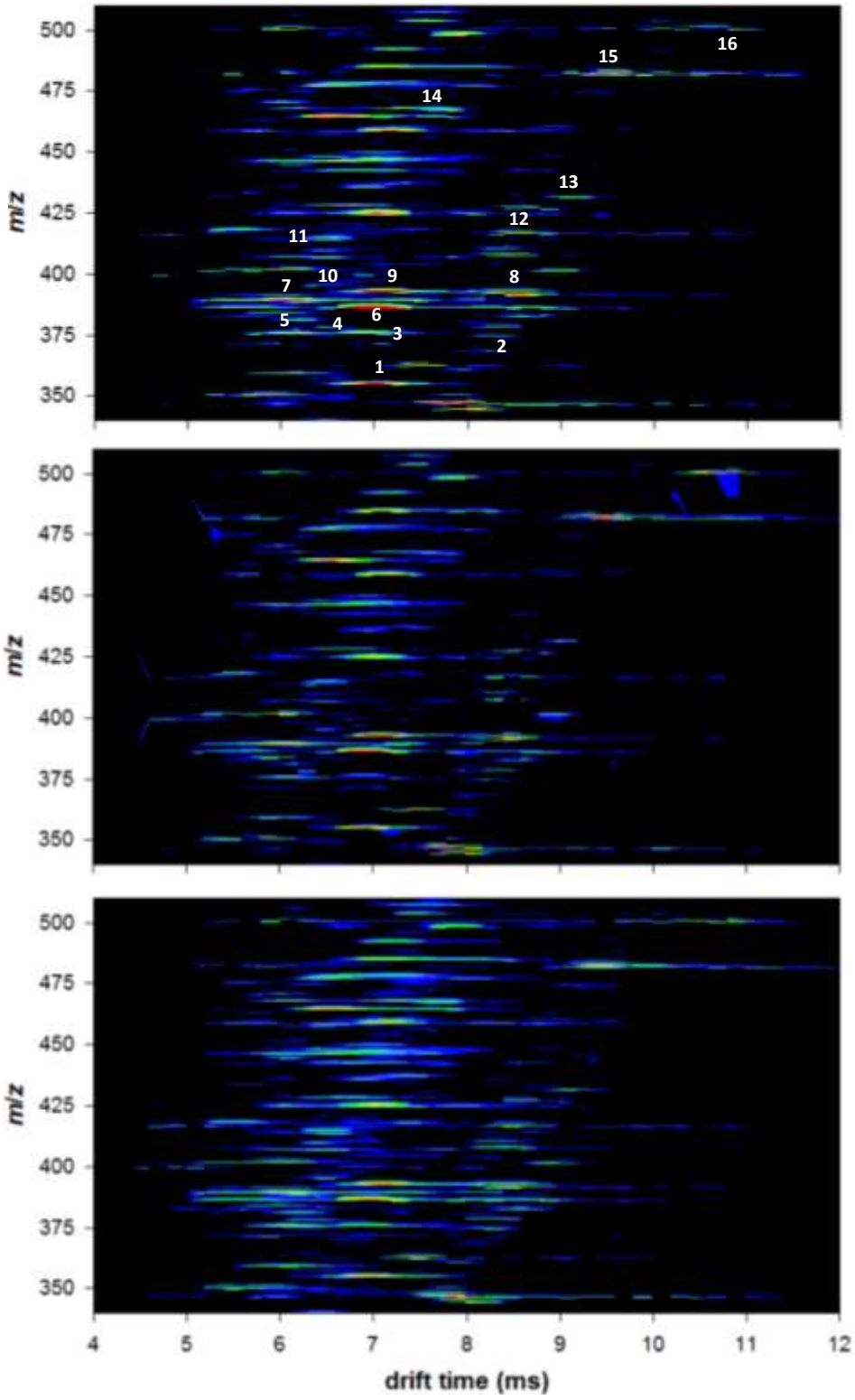

m/z
500
475
450
425
400
375
350
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
4
5
6
7
8
9
10
11
12
drift time (ms)

Figure S2

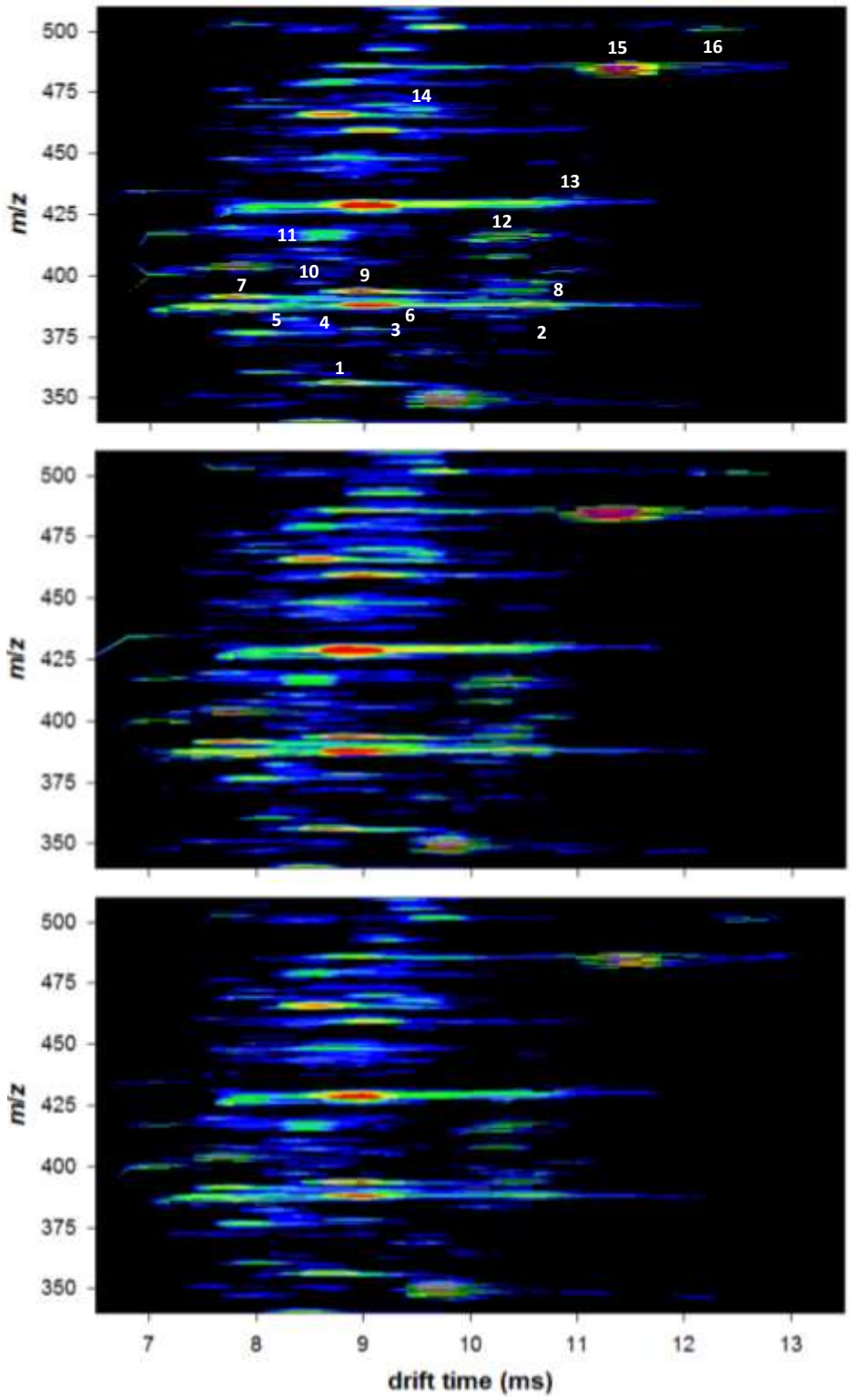

m/z
500
475
450
425
400
375
350
15
16
14
13
12
11
10
9
7
8
5
4
6
3
2
1
7
8
9
10
11
12
13
drift time (ms)

Figure S3

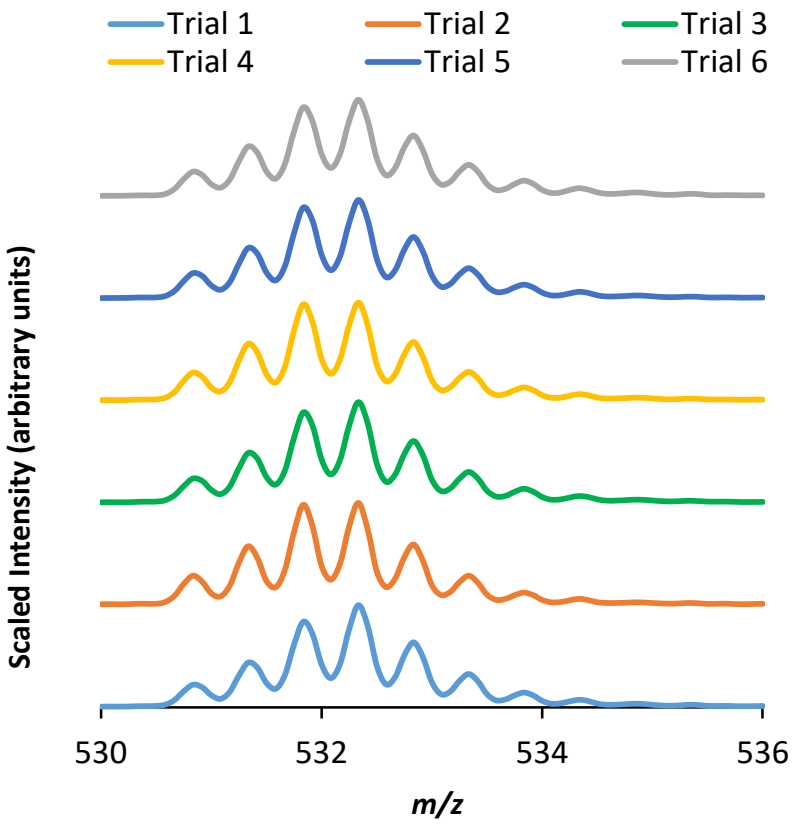

Trial 1
Trial 2
Trial 3
Trial 4
Trial 5
Trial 6
Scaled Intensity (arbitrary units)
530
532
534
536
m/z

Figure S4

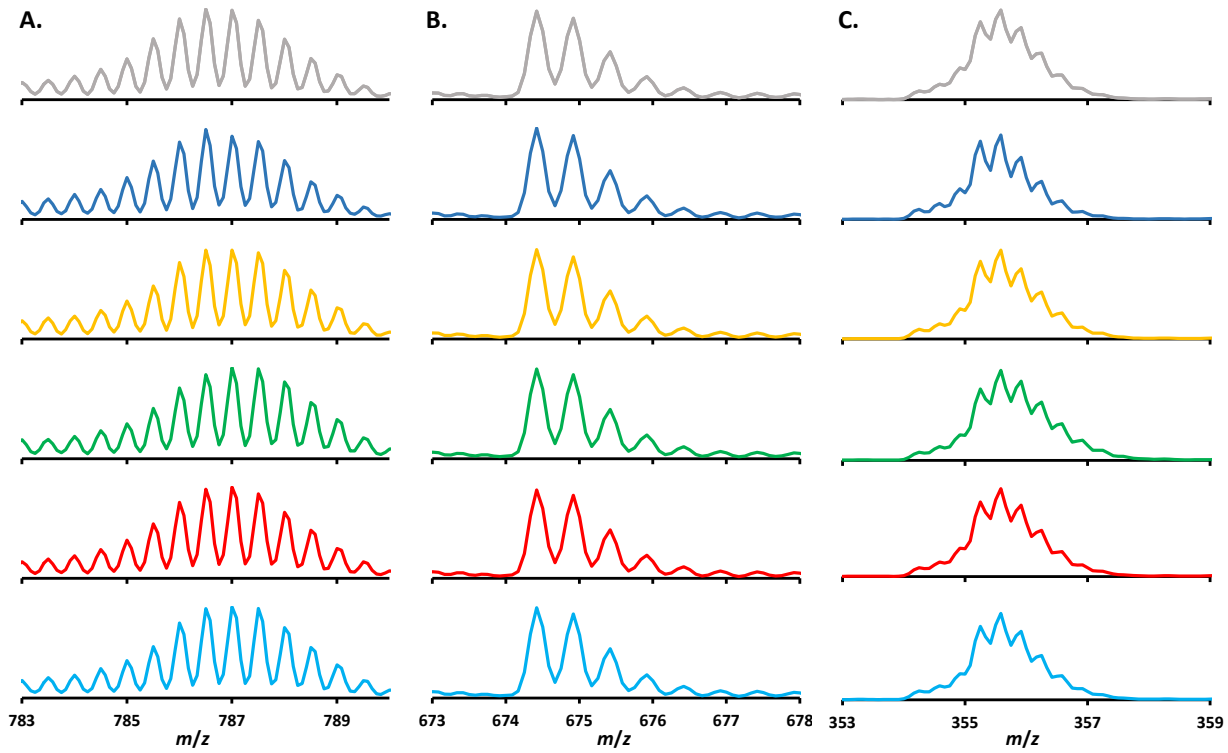

A.
B.
C.
783
785
787
789
m/z
673
674
675
676
677
678
m/z
353
355
357
359
m/z

Figure S5

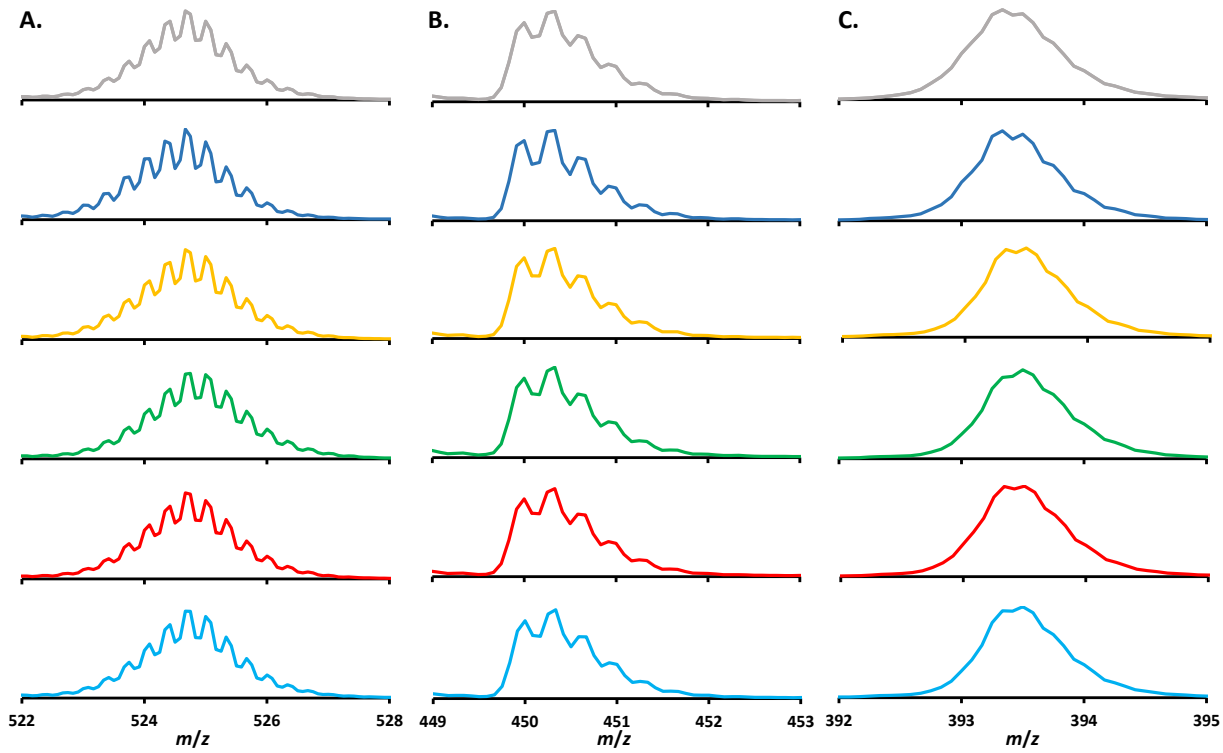


Figure S6

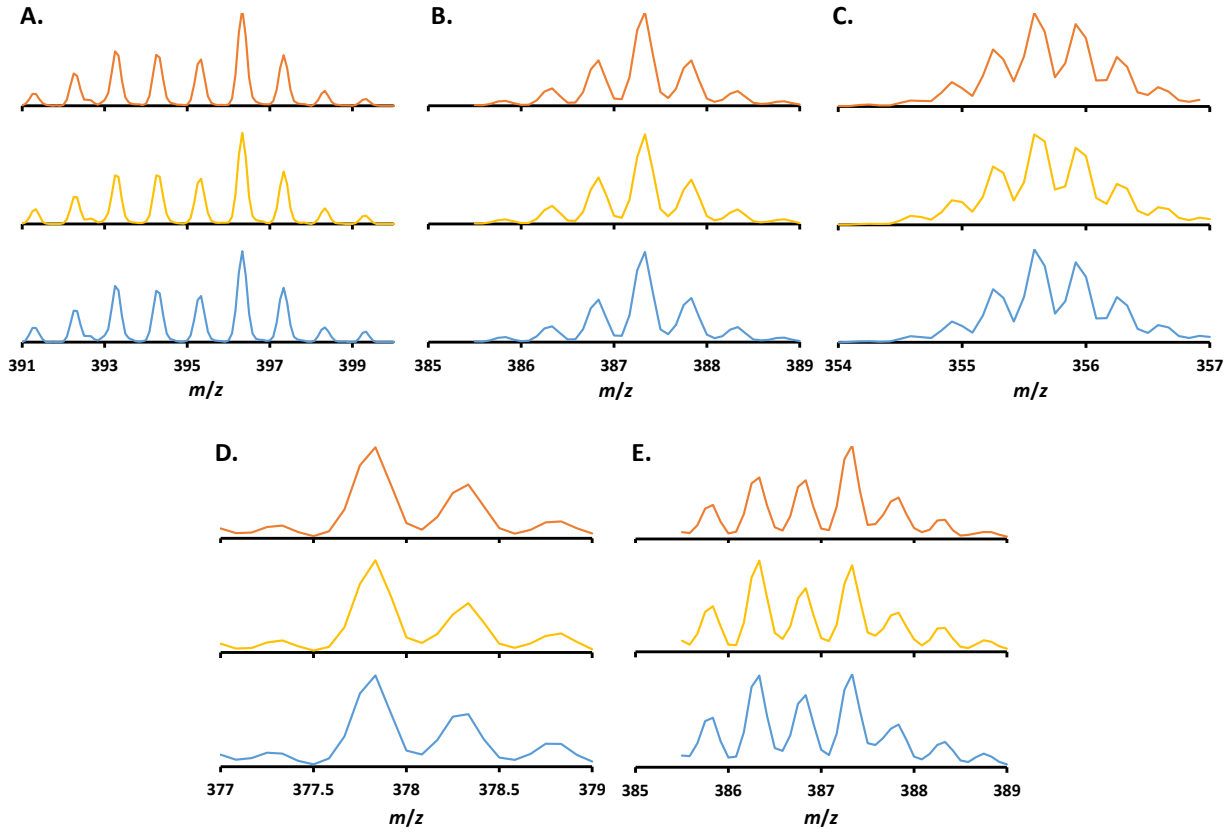

A.
B.
C.
391
393
395
397
399
385
386
387
388
389
354
355
356
357
m/z
m/z
m/z
D.
E.
377
377.5
378
378.5
379
385
386
387
388
389
m/z
m/z

Figure S7

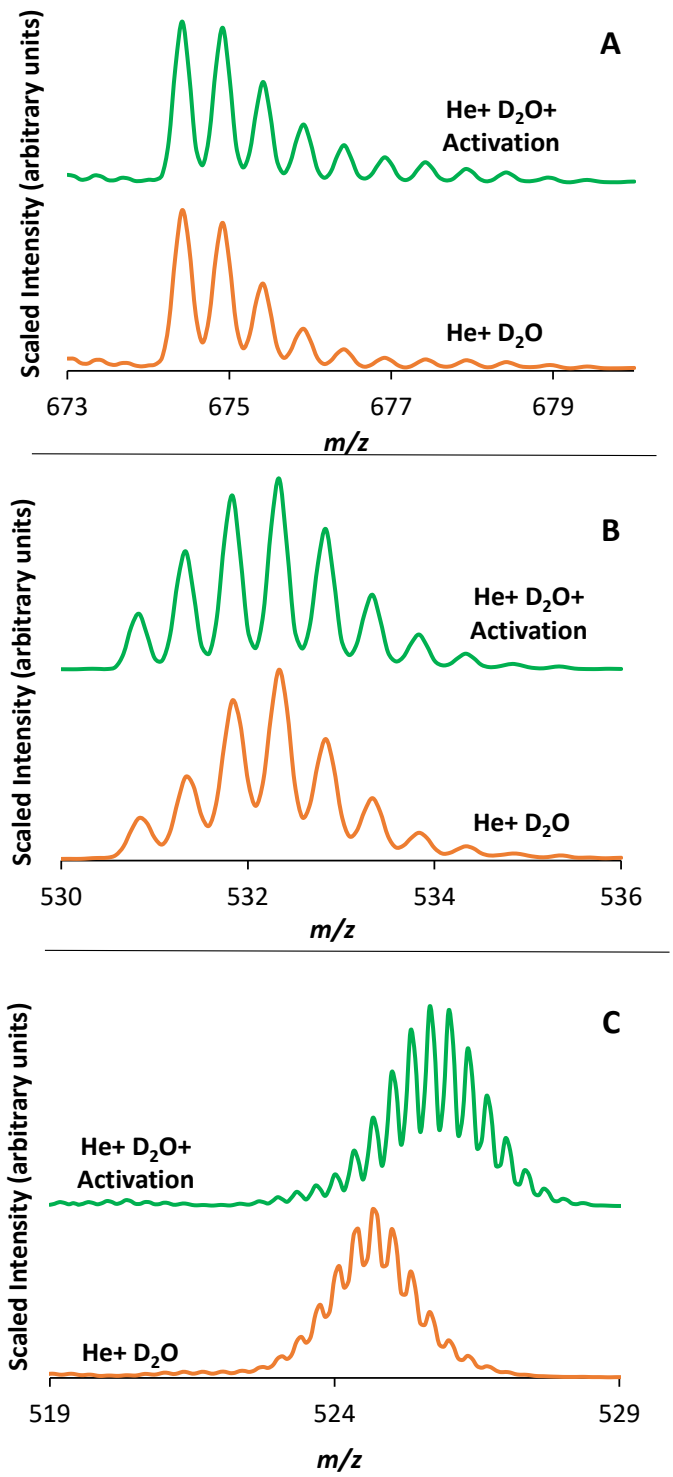

A
He+ D2O+
Activation
He+ D2O
Scaled Intensity (arbitrary units)
673
675
677
679
m/z
B
He+ D2O+
Activation
He+ D2O
Scaled Intensity (arbitrary units)
530
532
534
536
m/z
C
He+ D2O+
Activation
He+ D2O
Scaled Intensity (arbitrary units)
519
524
529
m/z

Figure S8

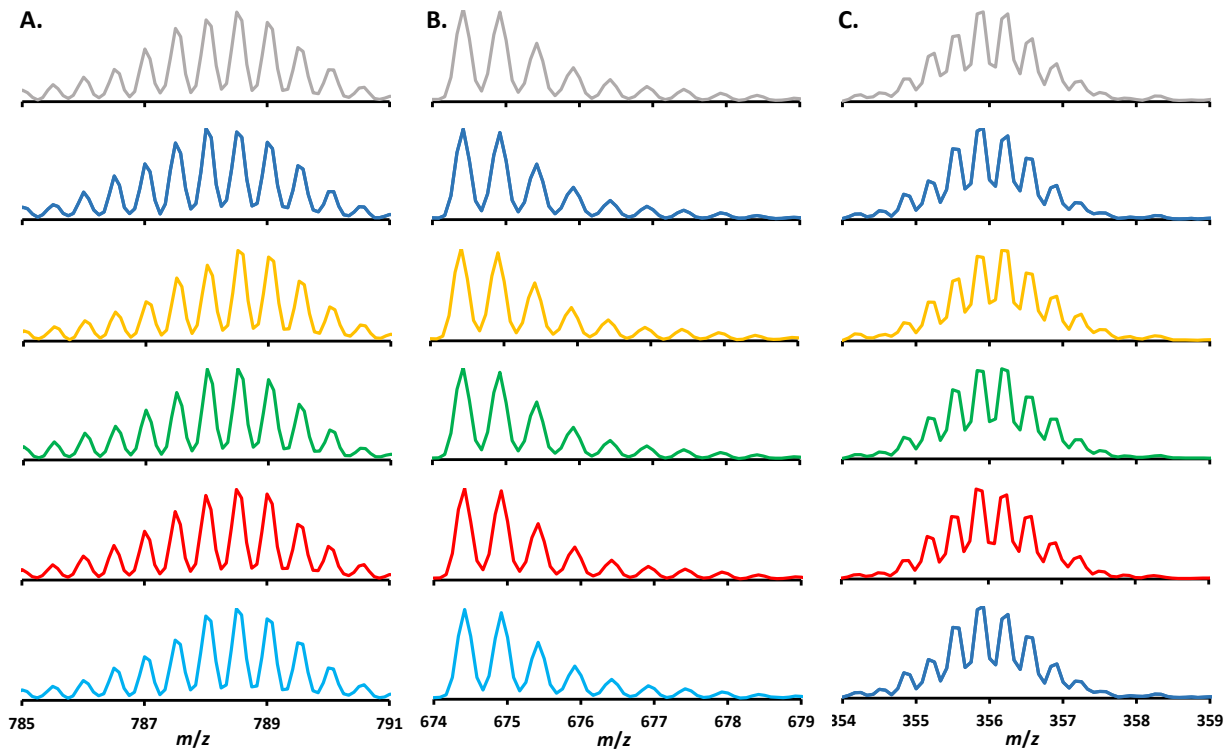

A.
B.
C.
785
787
789
791
m/z
674
675
676
677
678
679
m/z
354
355
356
357
358
359
m/z

Figure S9

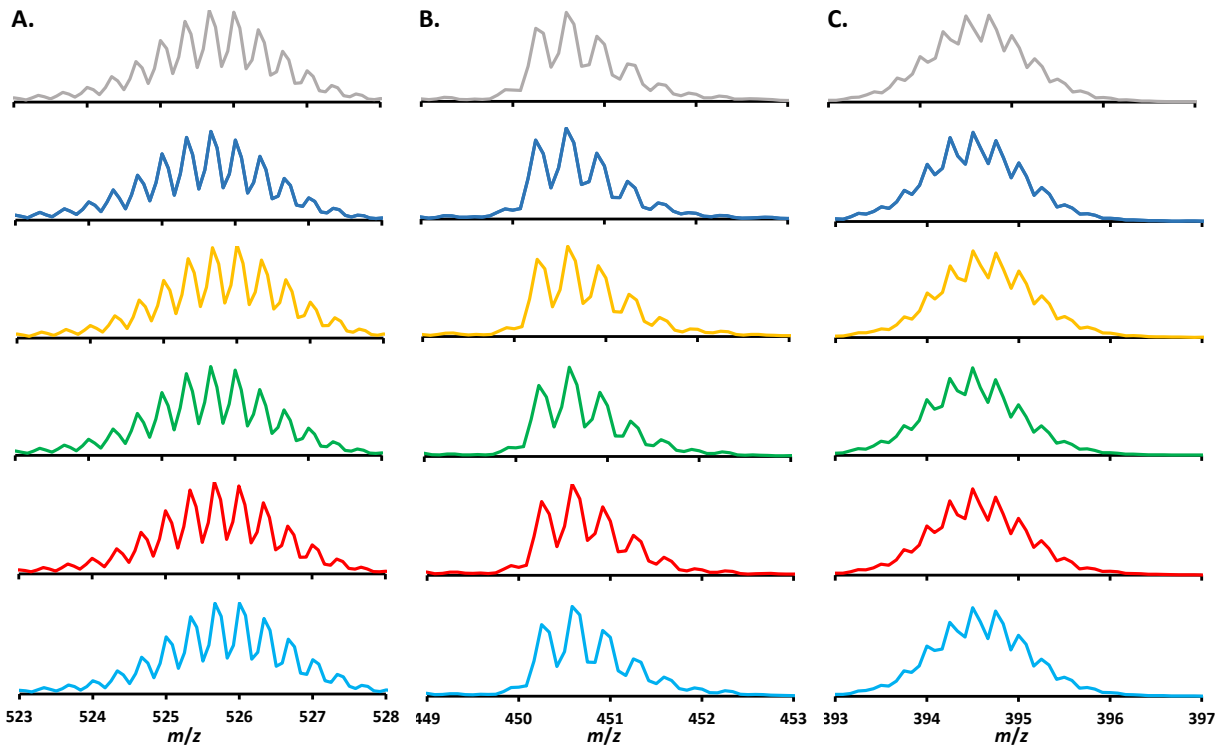

A.
B.
C.
523
524
525
526
527
528
449
450
451
452
453
393
394
395
396
397
m/z
m/z
m/z

Figure S10

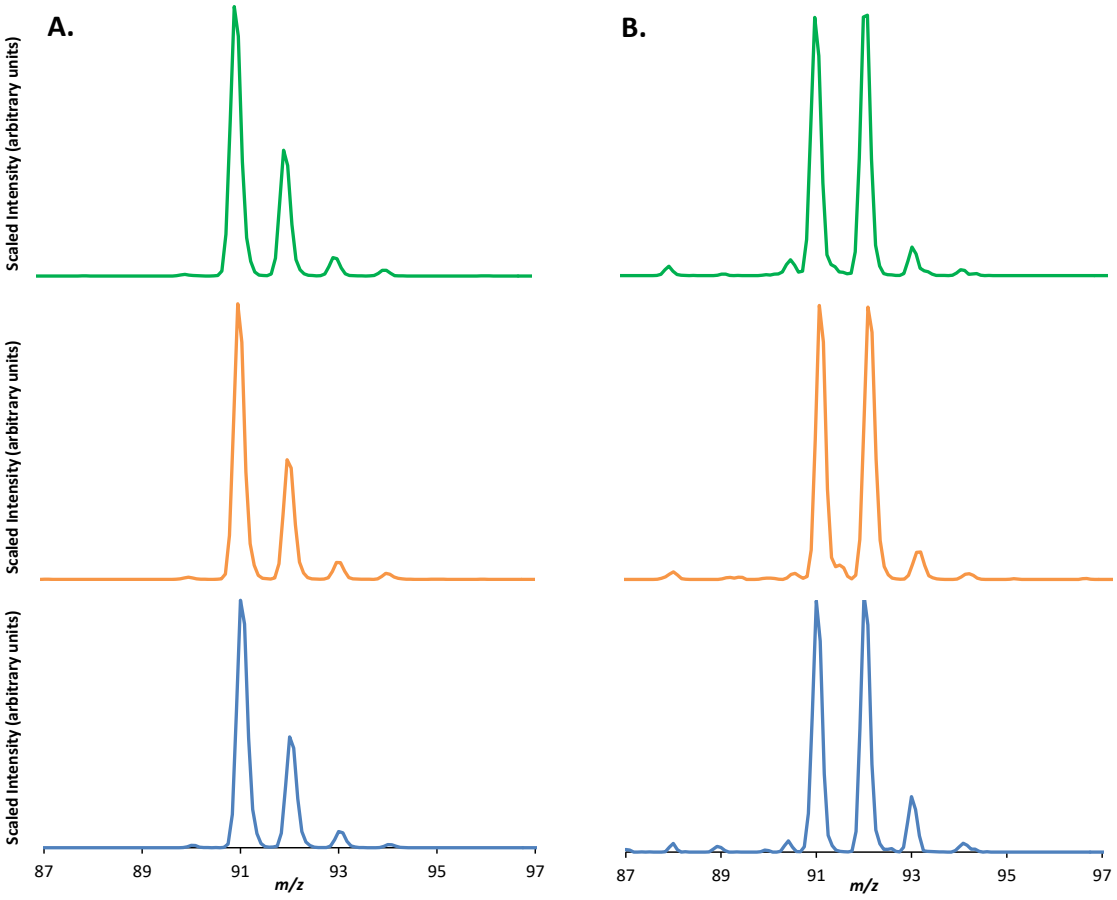

A.
B.
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
87
89
91
93
95
97
m/z
87
89
91
93
95
97
m/z

Figure S11

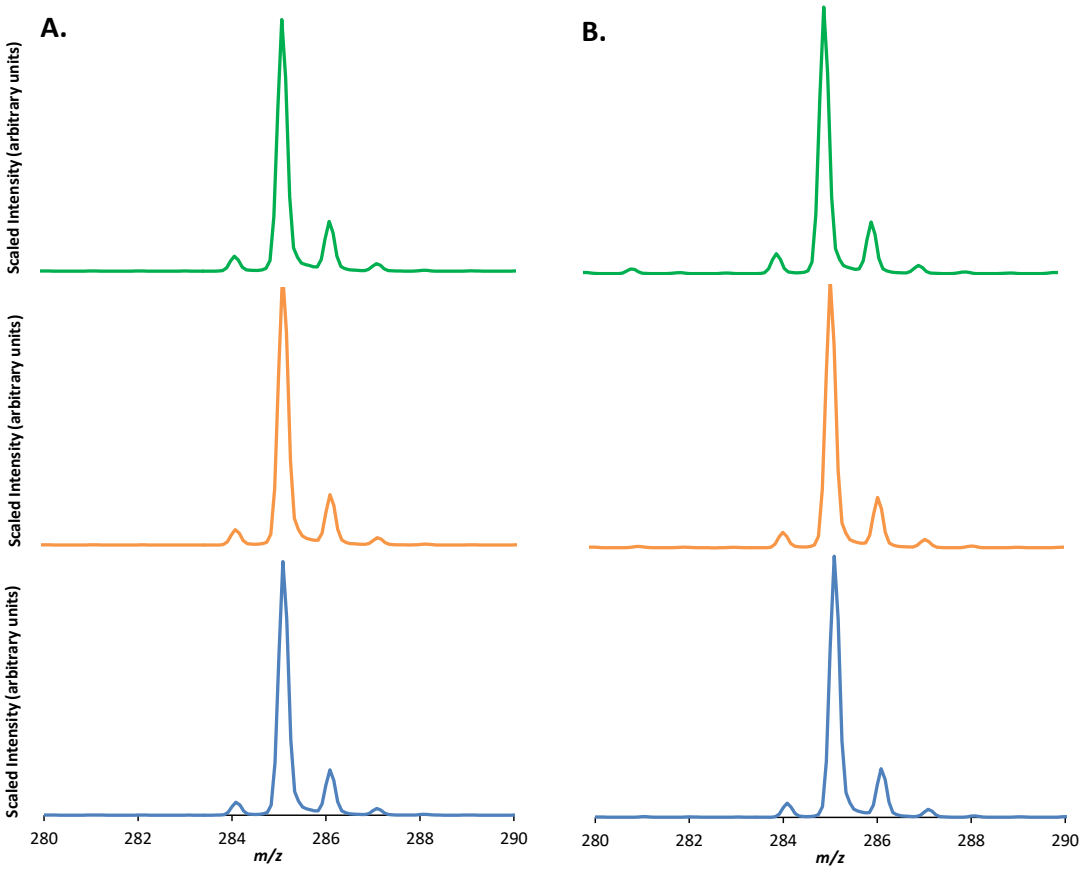

A.
B.
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
280
282
284
286
288
290
m/z
280
282
284
286
288
290
m/z

Figure S12

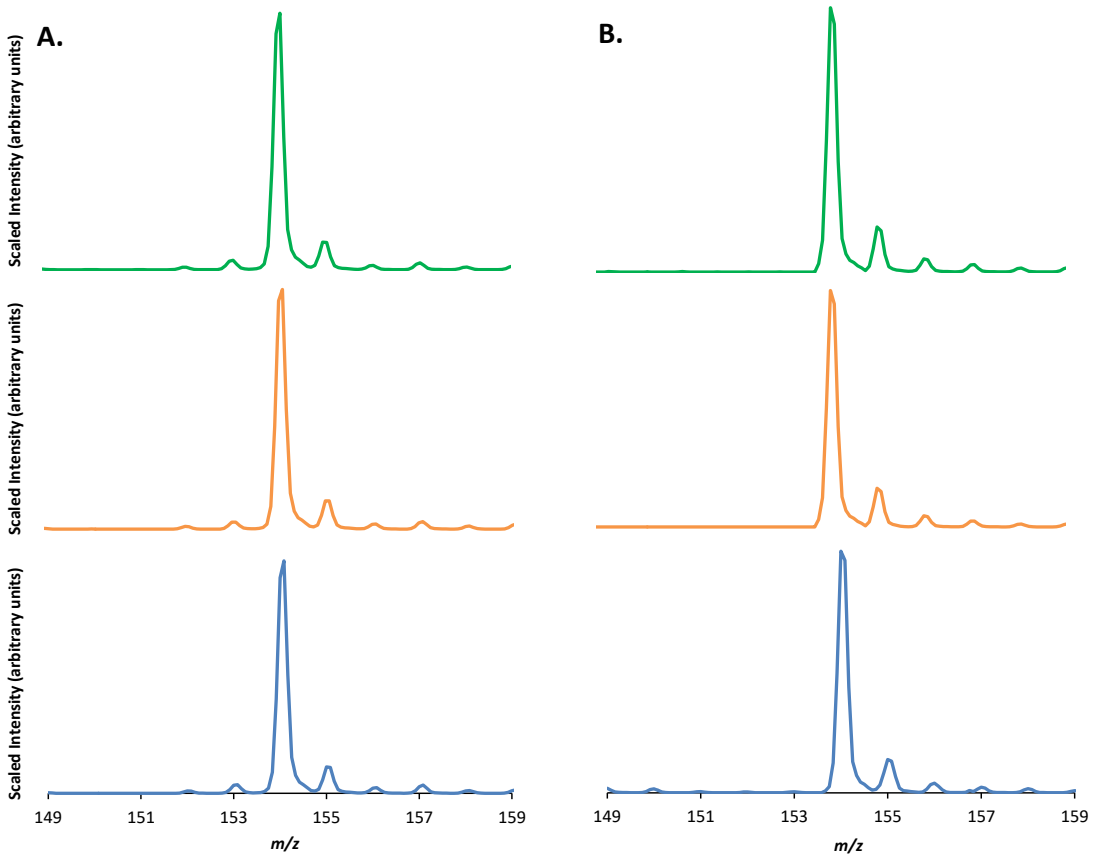

A.
B.
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
149
151
153
155
157
159
m/z
149
151
153
155
157
159
m/z

Figure S13

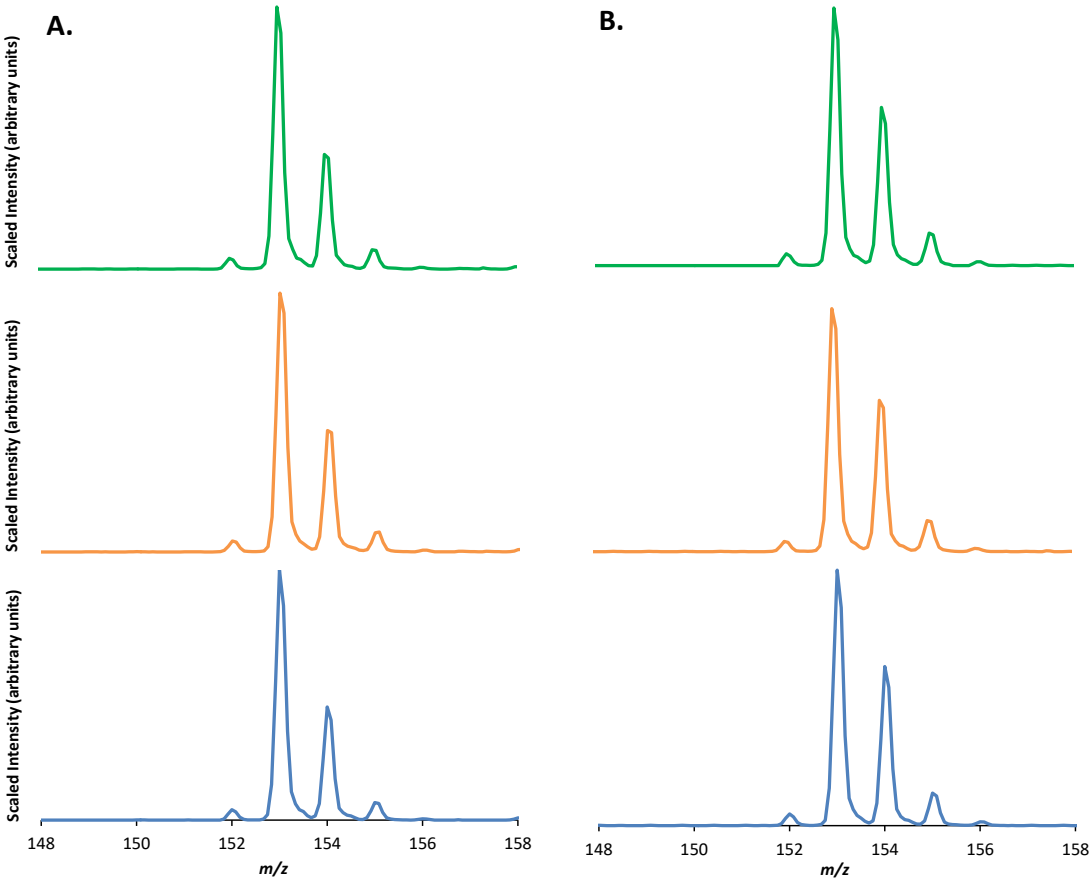

A.
B.
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
148
150
152
154
156
158
m/z
148
150
152
154
156
158
m/z

Figure S14

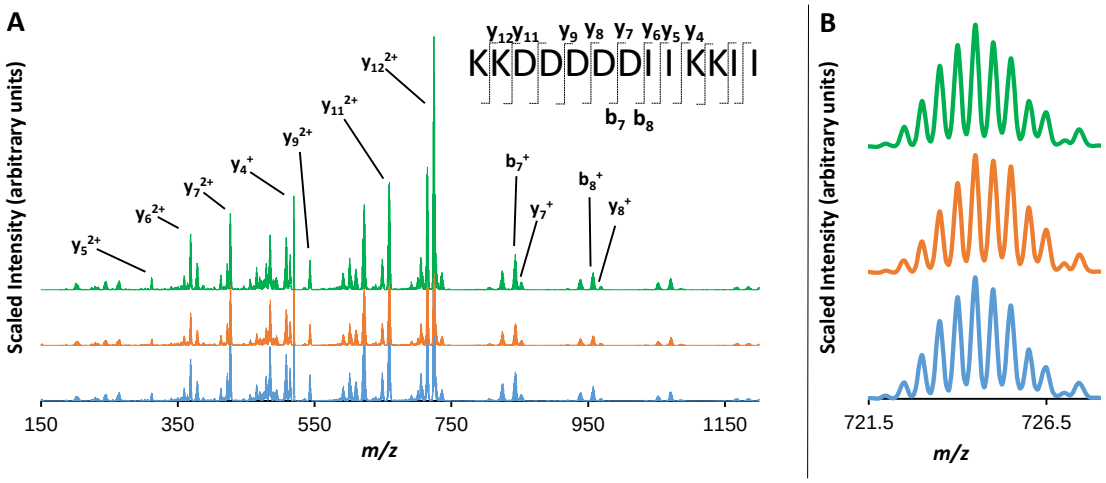

A
Scaled Intensity (arbitrary units)
150
350
550
750
950
1150
m/z
B
Scaled Intensity (arbitrary units)
721.5
726.5
m/z

Figure S15

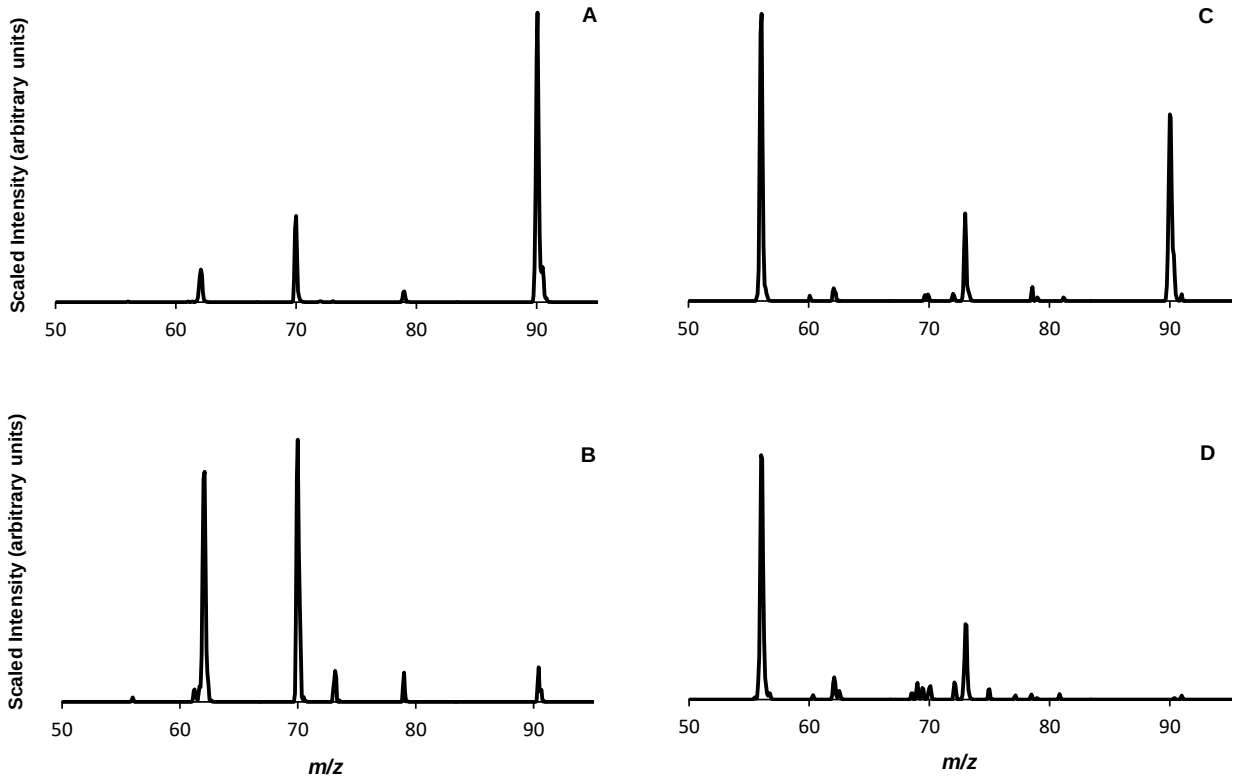

A
C
B
D
Scaled Intensity (arbitrary units)
Scaled Intensity (arbitrary units)
50
60
70
80
90
m/z
m/z

Figure S16