

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

Hossein Maleki¹, Megan M. Maurer¹, Nima Ronaghi and Stephen J. Valentine*

Department of Chemistry, West Virginia University, Morgantown, WV 26506

*E-mail correspondence to stephen.valentine@mail.wvu.edu; phone: (304) 293-4937; fax: (304) 293-4904

¹Authors contributed equally

Table of Contents:

Table S1. Drift times for ubiquitin peptide ions recorded using a random drift time selection process. The buffer gas was He.

Table S2. Drift times for ubiquitin peptide ions recorded using a sequential drift time selection process. The buffer gas was He.

Table S3. Drift times for ubiquitin peptide ions recorded using a sequential drift time selection process. The buffer gas was He and D₂O.

Table S4. Structures of the small molecule compounds used for HDX experiments and their reduced mobilities.

Figure S1. Two dimensional (2D) drift time, m/z plots for triplicate measurements of a ubiquitin digest mixture. The data was recorded for conditions employing He buffer gas and random mobility selection. Several dataset features are indicated by numbers and correspond to the assigned ions in Table S1, S2, and S3. See manuscript for details.

Figure S2. Two dimensional (2D) drift time, m/z plots for triplicate measurements of a ubiquitin digest mixture. The data was recorded for conditions employing He buffer gas and sequential mobility selection. Several dataset features are indicated by numbers and correspond to the assigned ions in Table S1, S2, and S3. See manuscript for details.

Figure S2. Two dimensional (2D) drift time, m/z plots for triplicate measurements of a ubiquitin digest mixture. The data was recorded for conditions employing He and D₂O buffer gas and sequential mobility selection. Several dataset features are indicated by numbers and correspond to the assigned ions in Table S1, S2, and S3. See manuscript for details.

Table of Contents (continued):

Figure S4. Isotopic distribution of $[M+2H]^{2+}$ bradykinin ions from 6 replicates of deuterium uptake. The RMSDs of the trials were less than 1%. See manuscript for details.

Figure S5. Isotopic distributions for $[M+2H]^{2+}$ KKD (A), $[M+2H]^{2+}$ substance P (B), and $[M+3H]^{3+}$ bradykinin (C) peptide ions. Six replicate analyses were performed for the RMSD calculations. RMSD values were less than 1% for these isotopic distributions.

Figure S6. Isotopic distributions for $[M+3H]^{3+}$ KKD (A), $[M+3H]^{3+}$ substance P (B), and $[M+4H]^{4+}$ KKD (C) peptide ions. Six replicate analyses were performed for the RMSD calculations. RMSD values were less than 1% for these isotopic distributions.

Figure S7. Isotopic distributions for mobility-resolved ubiquitin digest peptide ions. Data for $[DKE+H]^+$ (A), $[VLRLRGG+2H]^{2+}$ (B), $[QRLIFAGKQ+3H]^{3+}$ (C), $[HLVLRL+2H]^{2+}$ (D), and off-peak $[VLRLRGG+2H]^{2+}$ (E) peptide ions are shown. RMSD values are provided in the manuscript.

Figure S8. Model peptides showing different deuterium uptake patterns before and after collisional activation of the precursor ions in the drift tube. **A)** $[M+2H]^{2+}$ substance P ions. **B)** $[M+2H]^{2+}$ bradykinin ions. **C)** $[M+3H]^{3+}$ KKD peptide ions. Precursor and activated ions are represented by the orange (lower) and green (upper) traces, respectively.

Figure S9. Isotopic distributions for $[M+2H]^{2+}$ KKD (A), $[M+2H]^{2+}$ substance P (B), and $[M+3H]^{3+}$ bradykinin (C) peptide ions. These data were recorded under conditions in which the ions were collisionally activated to induce increased deuterium incorporation. Six replicate analyses were performed for the RMSD calculations. RMSD values were less than 1% for these isotopic distributions.

Figure S10. Isotopic distributions for $[M+3H]^{3+}$ KKD (A), $[M+3H]^{3+}$ substance P (B), and $[M+4H]^{4+}$ KKD (C) peptide ions. These data were recorded under conditions in which the ions were collisionally activated to induce increased deuterium incorporation. Six replicate analyses were performed for the RMSD calculations. RMSD values were less than 1% for these isotopic distributions.

Figure S11. Isotopic distributions for mobility-selected $[M+H]^+$ alanine ions from triplicate analyses. Data are shown for precursor (A) and collisionally activated (B) ions. RMSD values are provided in the manuscript.

Figure S12. Isotopic distributions for mobility-selected $[M+H]^+$ guanosine ions from triplicate analyses. Data are shown for precursor (A) and collisionally activated (B) ions. RMSD values are provided in the manuscript.

Table of Contents (continued):

Figure S13. Isotopic distributions for mobility-selected $[M+H]^+$ dopamine ions from triplicate analyses. Data are shown for precursor (A) and collisionally activated (B) ions. RMSD values are provided in the manuscript.

Figure S14. Isotopic distributions for mobility-selected $[M+H]^+$ acetaminophen ions from triplicate analyses. Data are shown for precursor (A) and collisionally activated (B) ions. RMSD values are provided in the manuscript.

Figure S15. A) Fragmentation spectra for $[M+3H]^{3+}$ KKD peptide ions after HDX and collisional activation. The precursor ion was drift selected at 6.7 ms and fragmented at 40 NCE. Several fragment ions have been labeled. B) Isotopic distribution reproducibility of the y_{12}^{2+} fragment ion.

Figure S16. CID spectra for alanine at A) 15 NCE and B) 25 NCE. CID spectra for a feature with nominal m/z value of 90 within the bovine heart metabolite extract sample at C) 15 NCE and D) 25 NCE.

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

Peptide Ion ^a	<i>m/z</i> ^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

^aPeptide ion assignments based on *m/z* matches to monoisotopic peaks and charge (obtained from isotopic distribution)

^bExperimental *m/z* values for monoisotopic peaks from the 2D datasets

^cIon drift times in ms as obtained from 2D dataset centroids for the assigned ions

^dAverage ion drift times in ms for the three experimental runs

^eCoefficients 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	<i>m/z</i> ^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

^aPeptide ion assignments based on *m/z* matches to monoisotopic peaks and charge (obtained from isotopic distribution)

^bExperimental *m/z* values for monoisotopic peaks from the 2D datasets

^cIon drift times in ms as obtained from 2D dataset centroids for the assigned ions

^dAverage ion drift times in ms for the three experimental runs

^eCoefficients of variation for the drift times expressed as a percentage

Table S3. Drift times for D₂O-exposed peptide ions from experimental runs employing sequential mobility selection.

Peptide Ion ^a	<i>m/z</i> ^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

^aPeptide ion assignments based on *m/z* matches to monoisotopic peaks and charge (obtained from isotopic distribution)

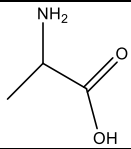
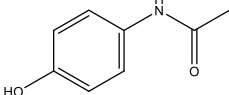
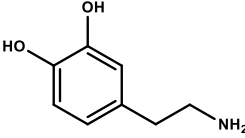
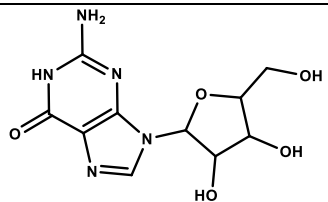
^bExperimental *m/z* values for monoisotopic peaks from the 2D datasets

^cIon drift times in ms as obtained from 2D dataset centroids for the assigned ions

^dAverage ion drift times in ms for the three experimental runs

^eCoefficients 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.

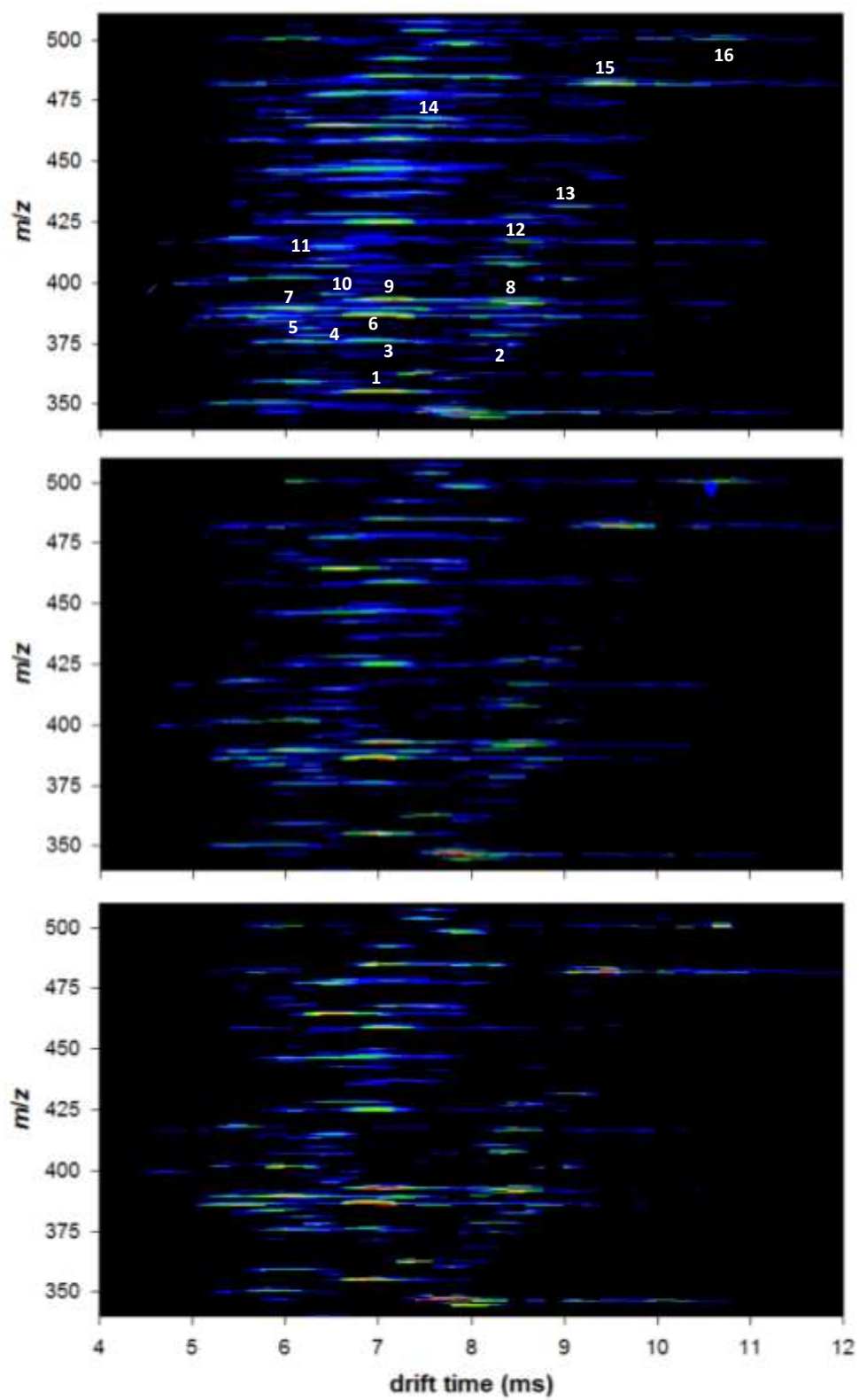


Figure S1

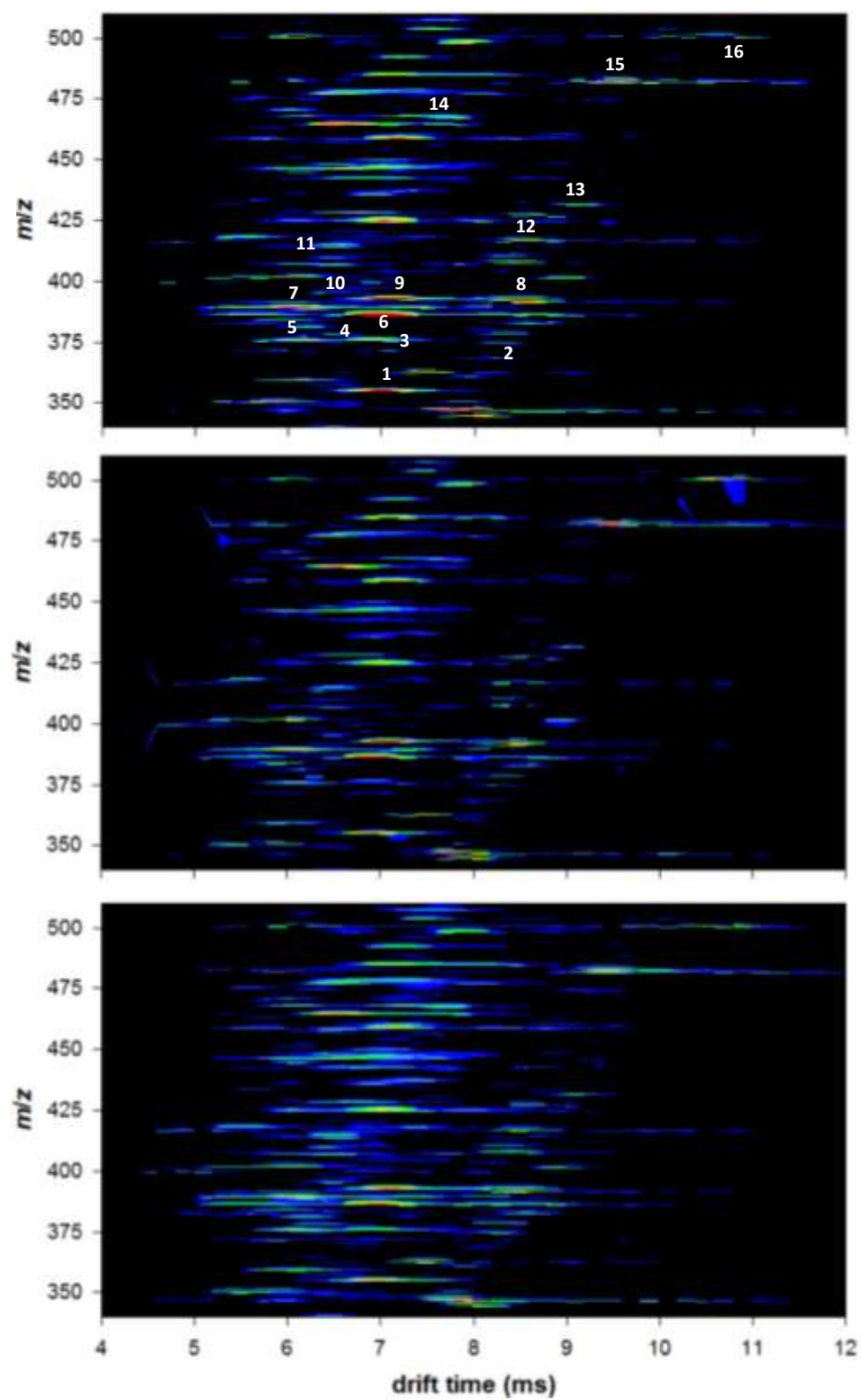


Figure S2

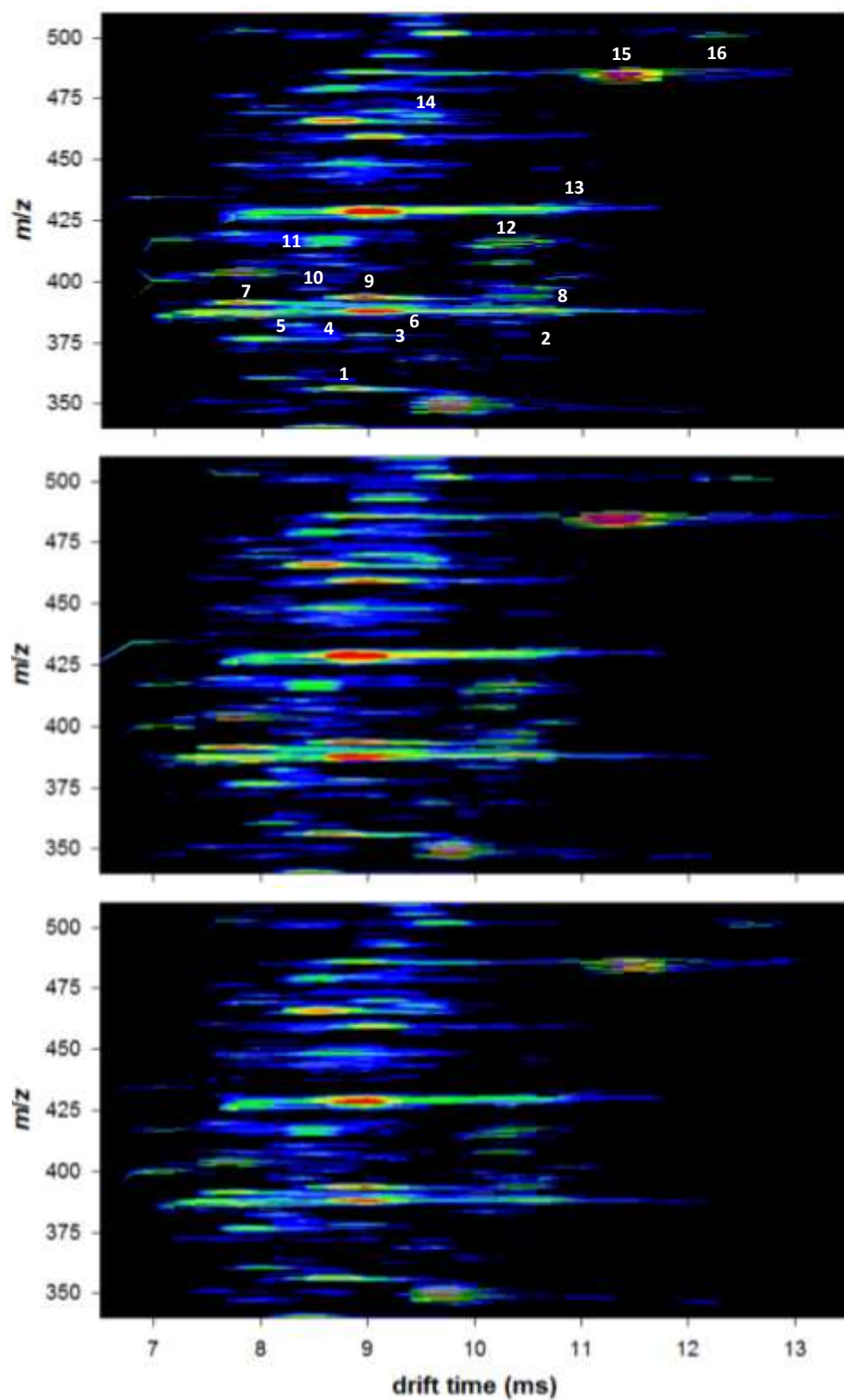


Figure S3

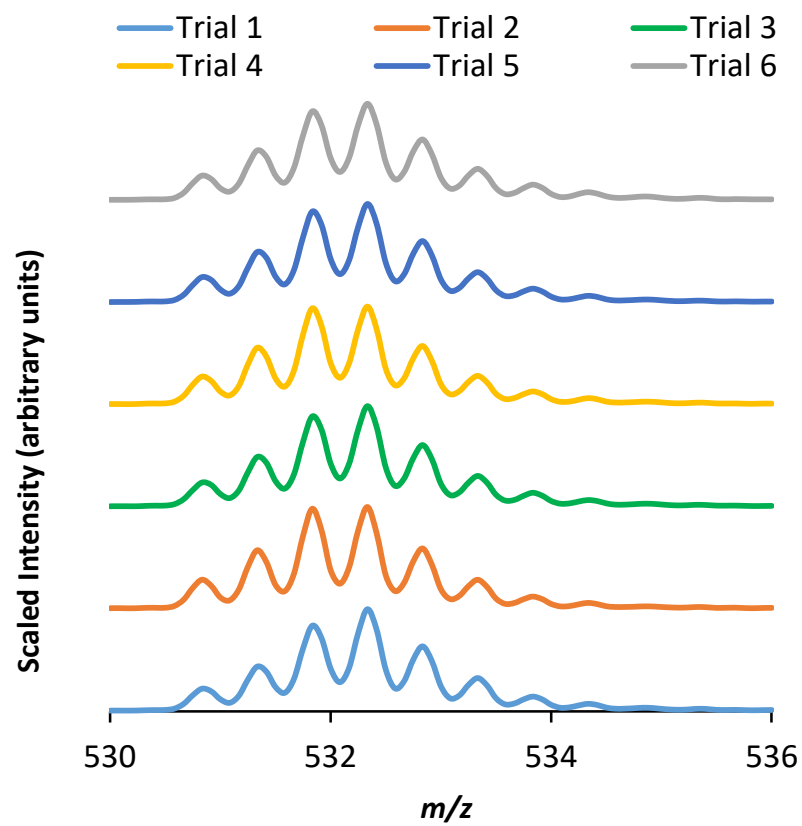


Figure S4

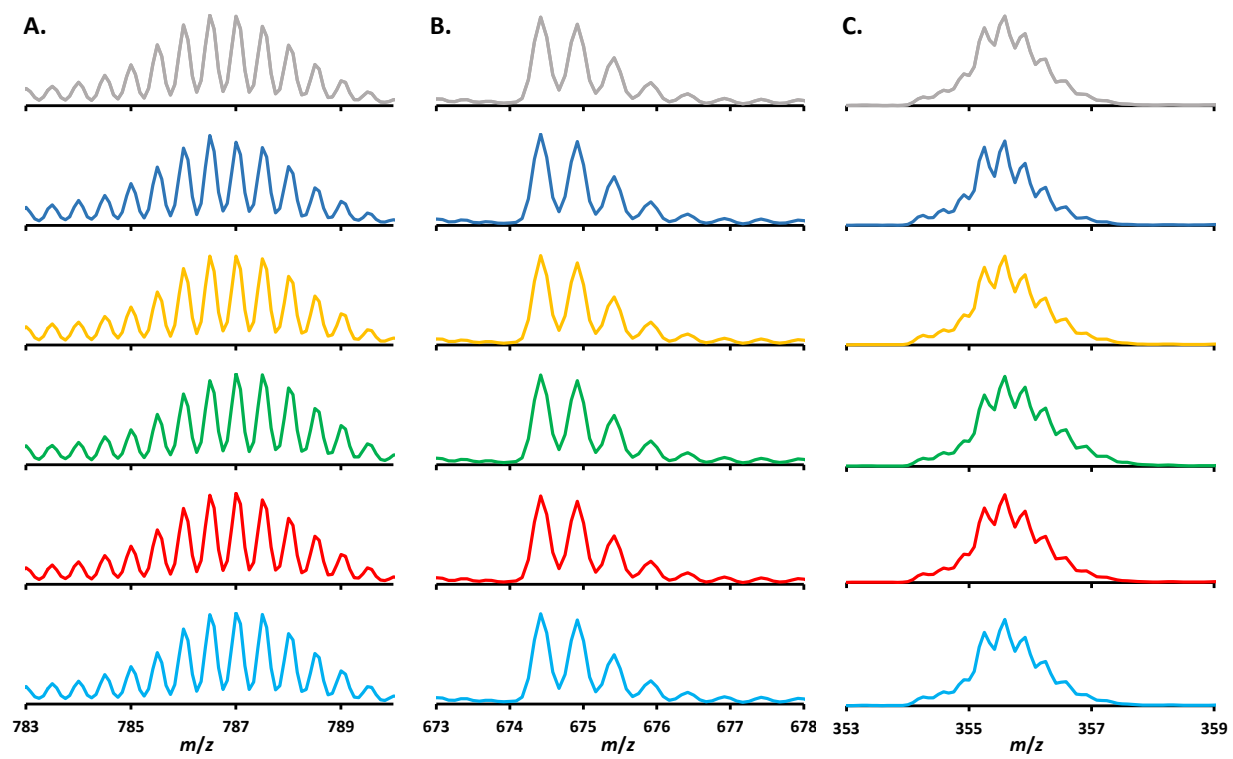


Figure S5

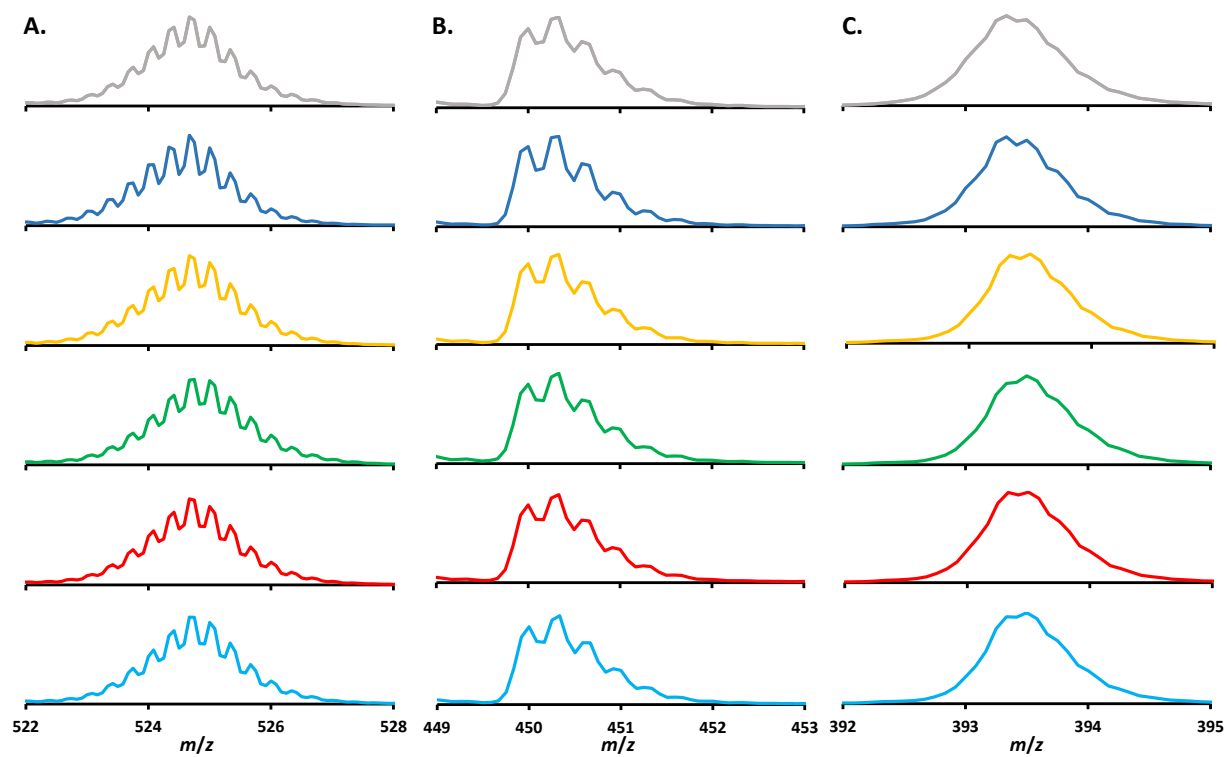


Figure S6

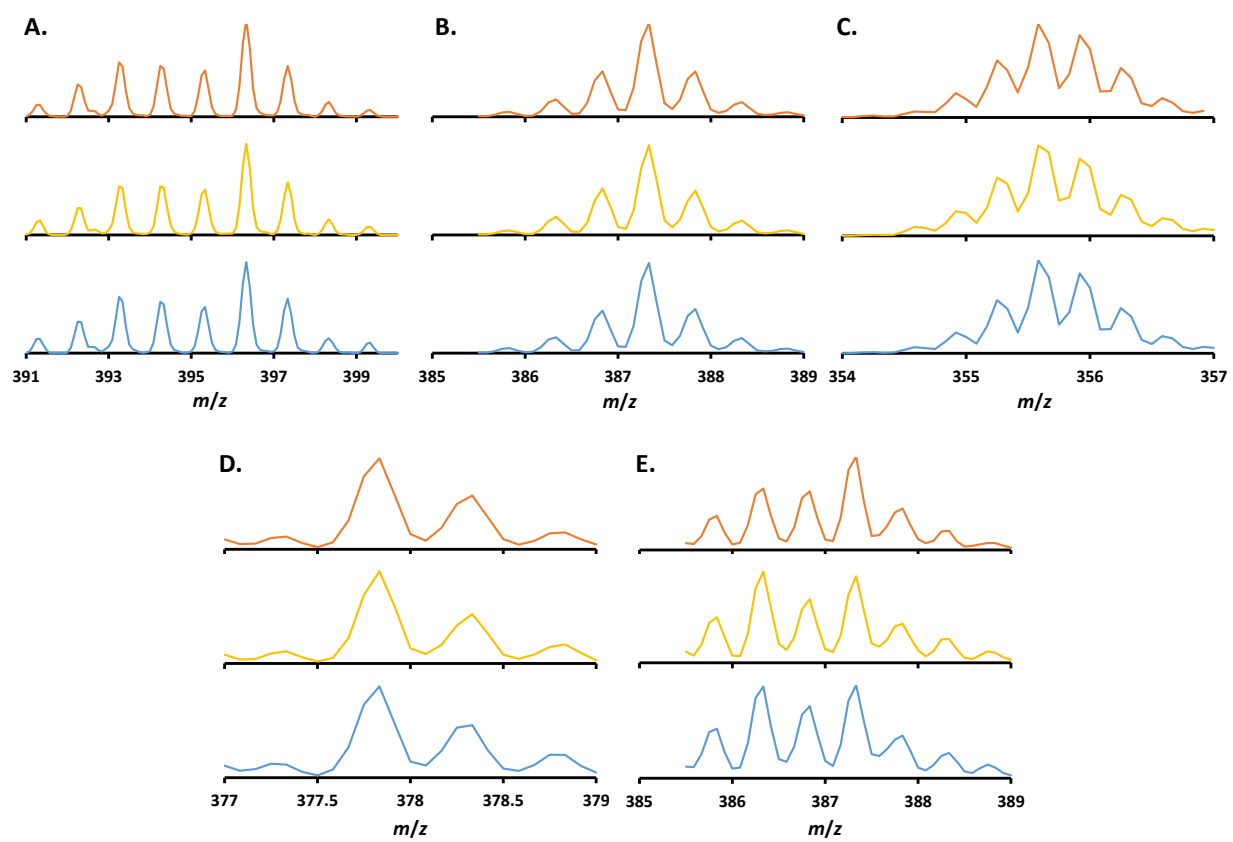


Figure S7

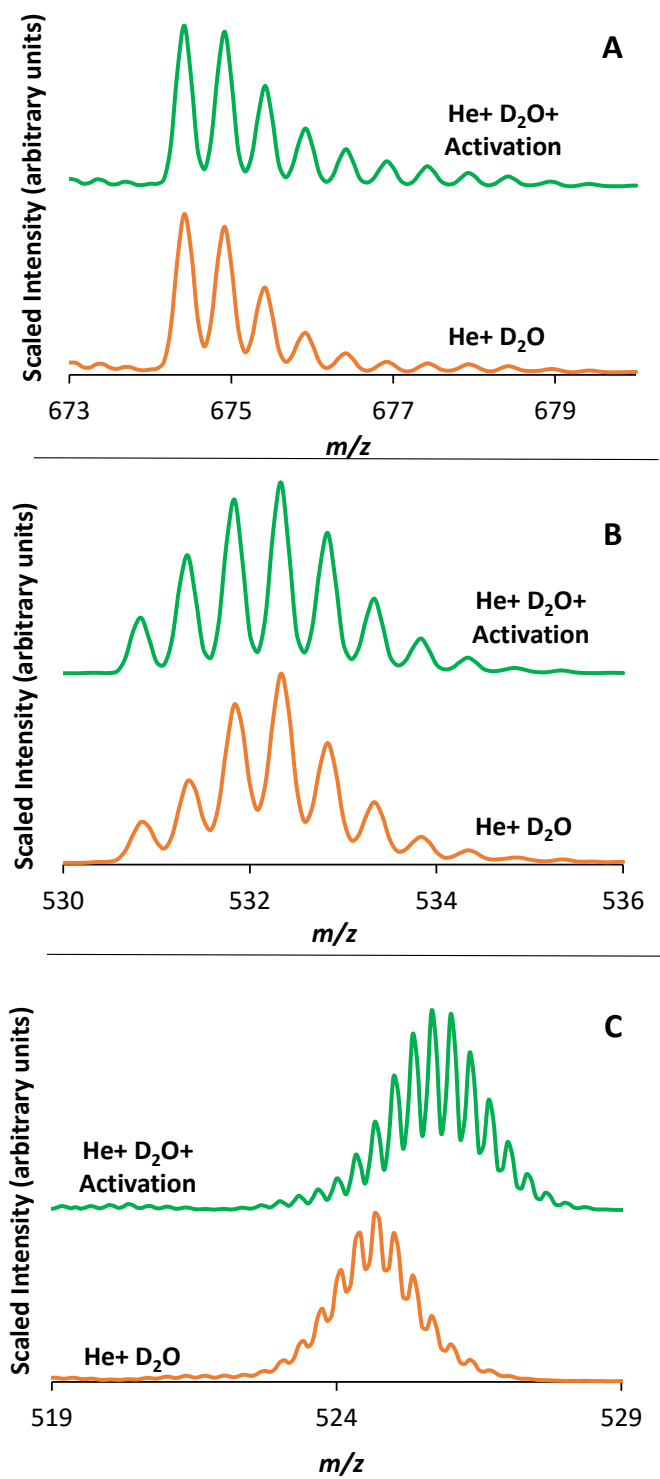


Figure S8

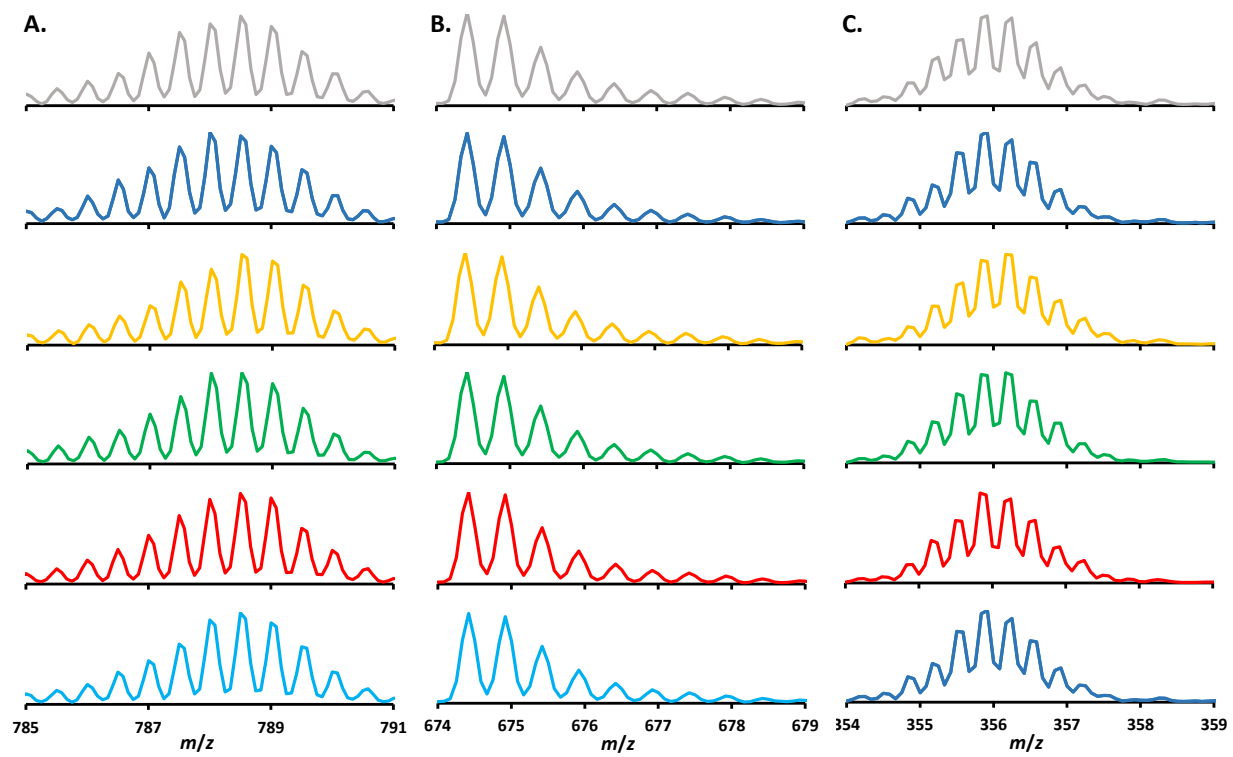


Figure S9

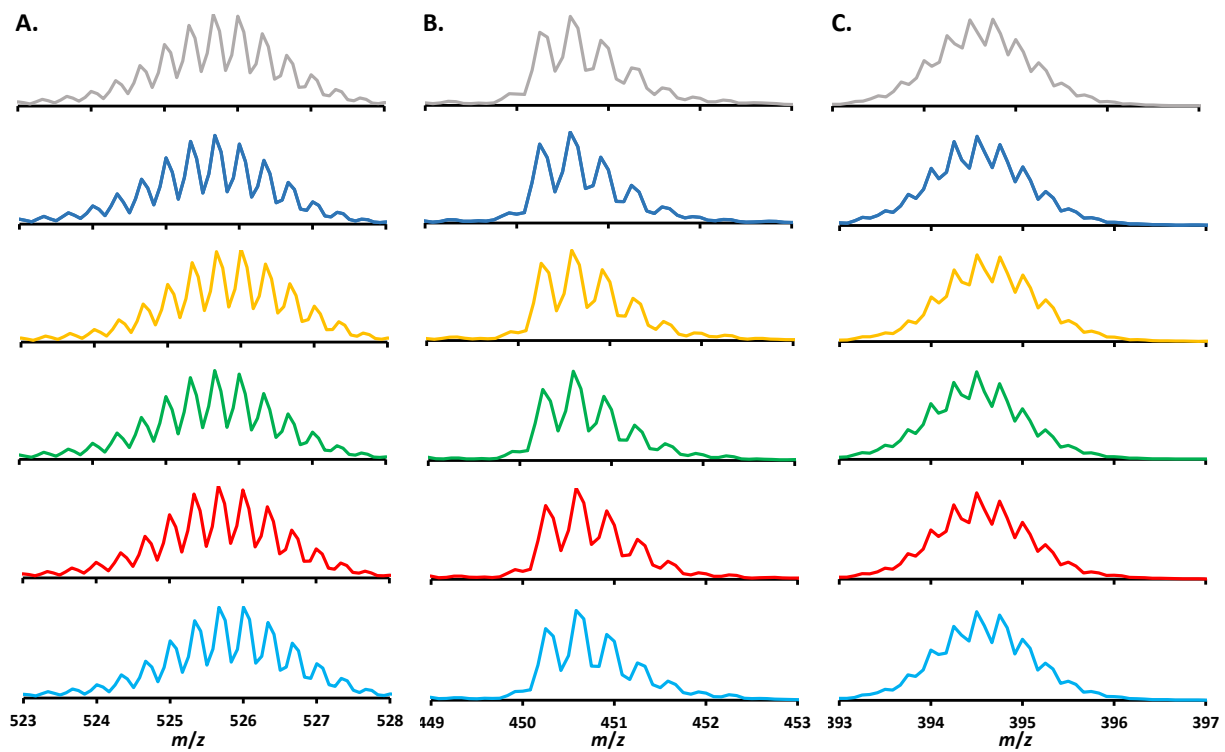


Figure S10

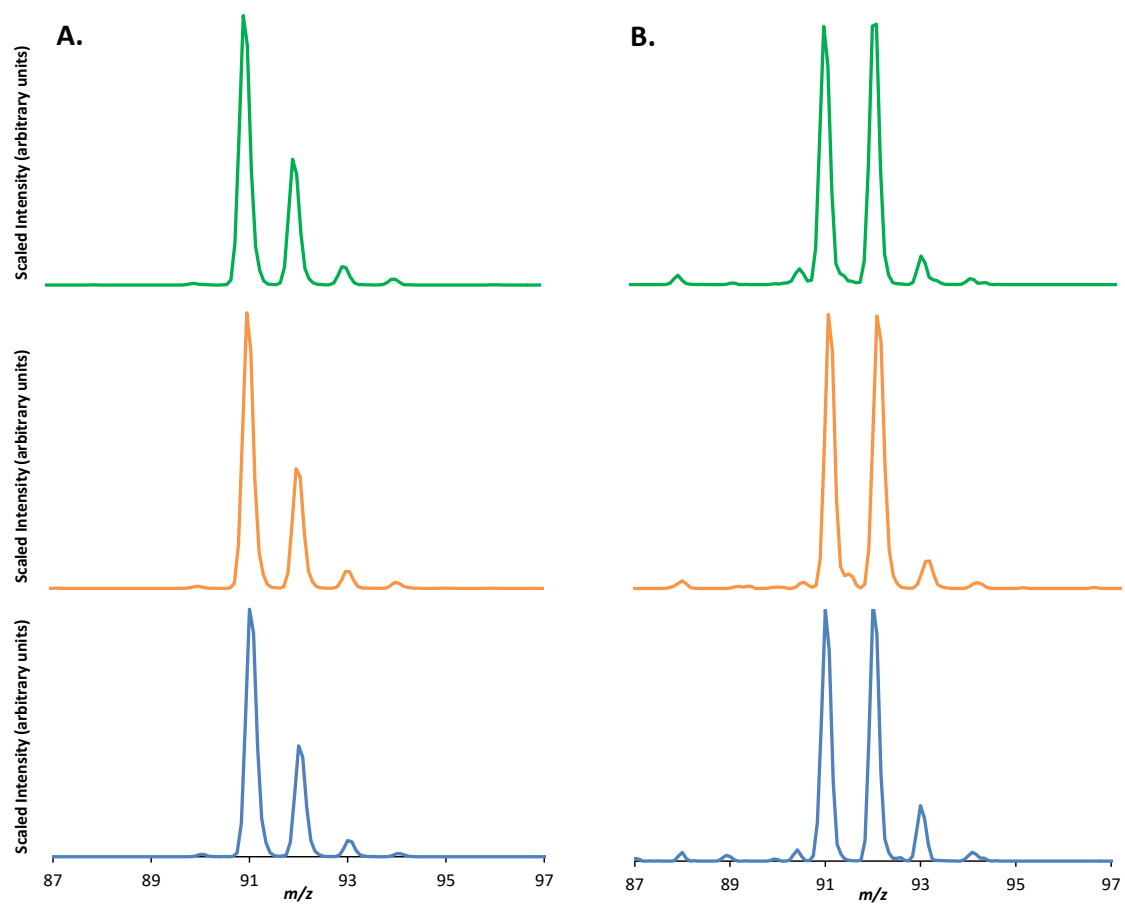


Figure S11

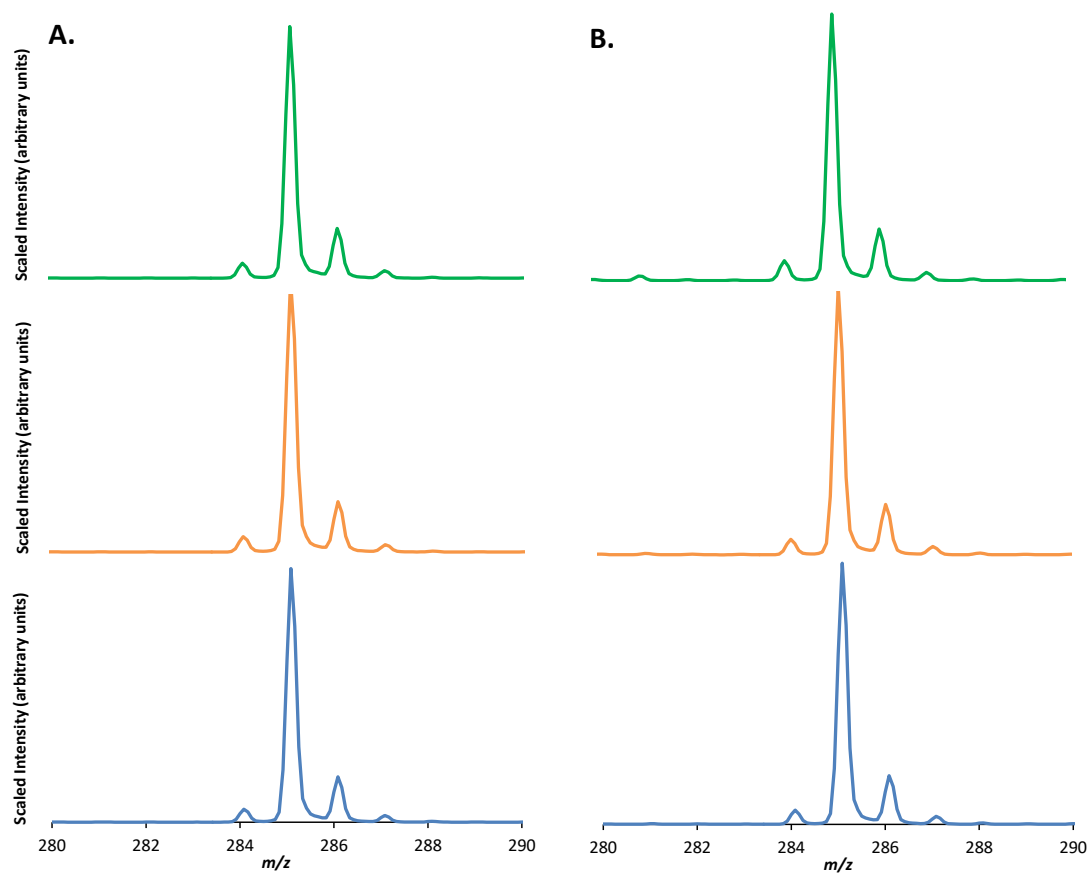


Figure S12

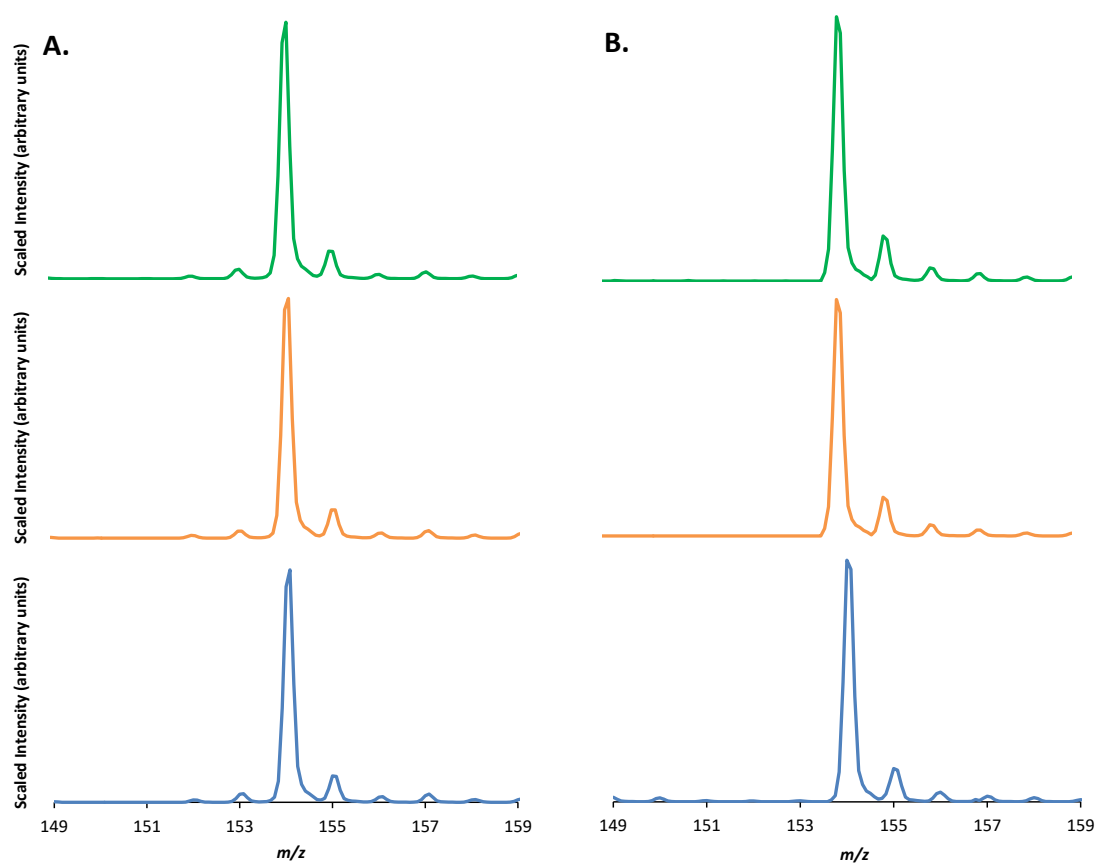


Figure S13

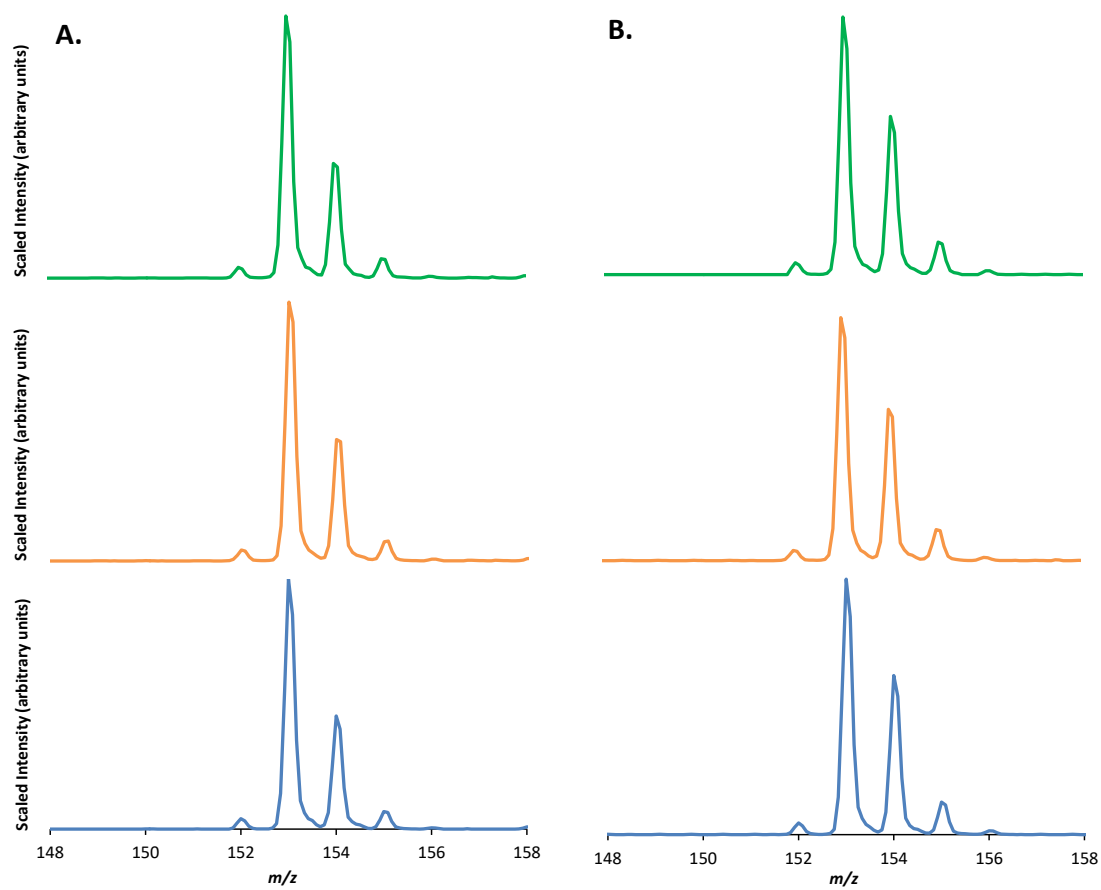


Figure S14

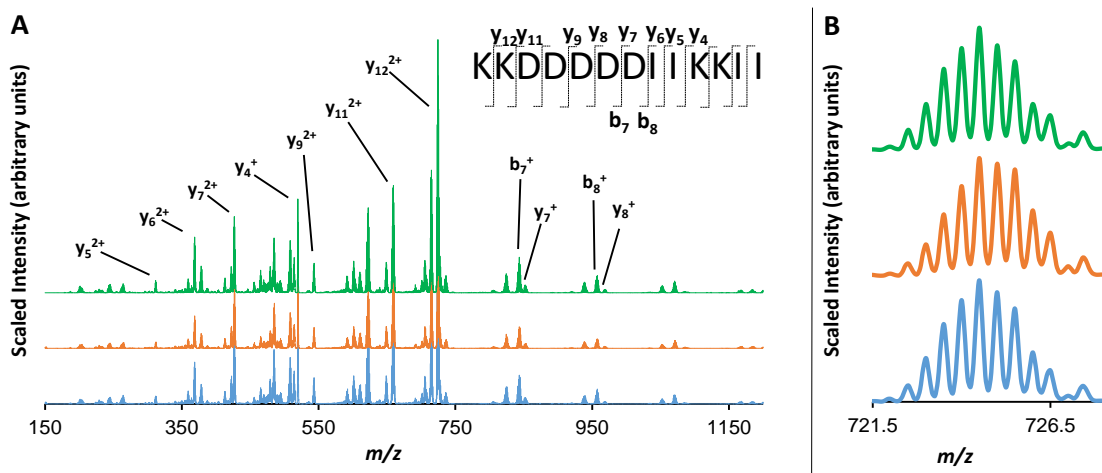


Figure S15

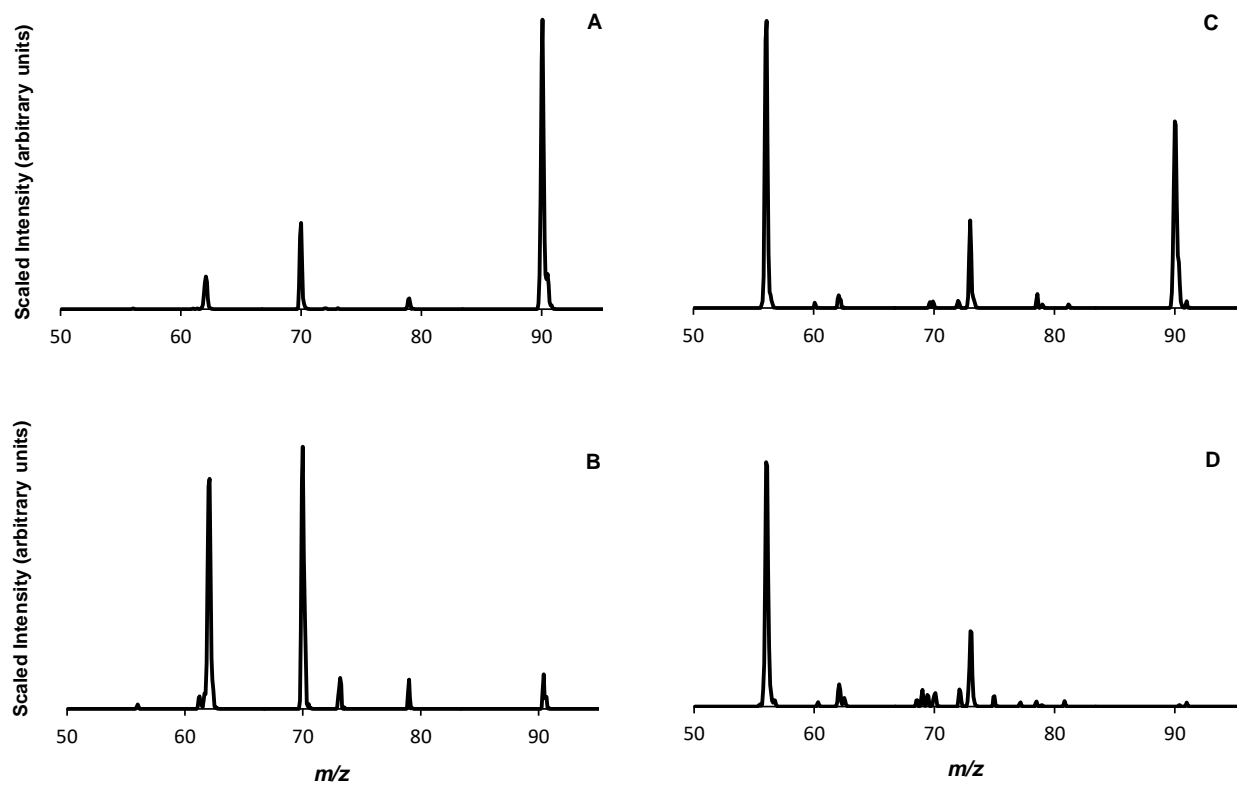


Figure S16