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WA20699

A Method for Detecting Post-Translational Modifications in Intact Proteins and Peptides

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Waters Corporation. Milford, MA

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Carbamylation, Oxidation, Deamidation

Occur

- *In Vivo*
- *In Sample Preparation*
  - *Upstream and Downstream Processing*
- *In Storage over time*

- *Markers of Disease State*
- *Affect Efficacy of Proteins and Peptides*
- *Affect Identification and Characterization of proteins and peptides*

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Methodological Approach for Characterization of

- *Carbamylation, Oxidation, Deamidation*

- *LC/UV Proteins*
- *LC/MS Proteins*

- *LC/UV Peptides*
- *LC/MS Peptides*
- *LC/MS/MS*

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Instrument Methodology for Proteins and Peptides

- *Waters ZQ™ 4000 or a Micromass Q-ToF 2™ MS*
- *Waters Alliance 2790 or 2690 LC*
- *Positive electrospray ionization*
- *Capillary Voltage = 3.3 kV*
- *Extraction Cone Voltage between 20-25 V Protein / 25-35 Peptide*
- *Source Temperature = 150°C,*
- *Desolvation Temperature = 400°C*
- *Cone Gas Flow = 50 L/Hr, Desolvation Gas Flow = 500 L/Hr.*
- *MS/MS collision engergy between 30-35*
- *MassLynx™ v3.5 Control*

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Protein Separations Methodology

Delta-Pak™ or Symmetry® *300 columns*

C_{18}; 4.6 x 50mm; 5 micron

200Å or 300Å Pores

Gradient from 15% B to 80% B in 15 min

A: 0.1% formic acid in water

B: 0.1% formic acid in acetonitrile

Flow rate of 0.5 mL/min Split

0.2 mL/min into the mass spectrometer

0.3mL/min being diverted into the 996 PDA detector.

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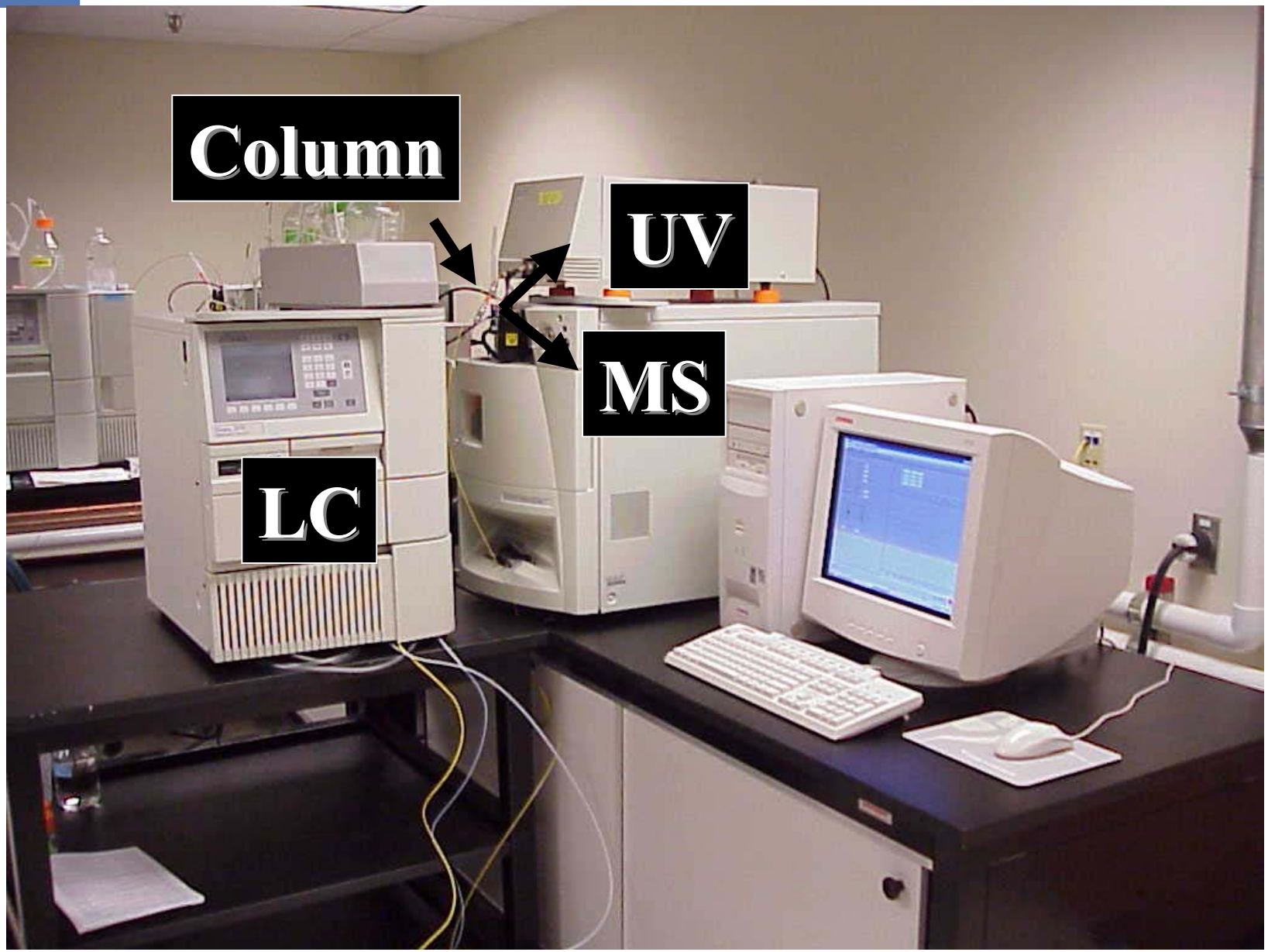

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Peptide Separations Methodology

RapiGest™ *Digestion Aid*
Atlantis™ *columns*

C_{18}; 4.6 x 50mm; 3.5 micron
100Å Pores

Gradient from 0% B to 40% B in 45 min

A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile
Flow rate of 0.75 mL/min Split

0.2 mL/min into the mass spectrometer
0.55mL/min being diverted into the 996 PDA detector.

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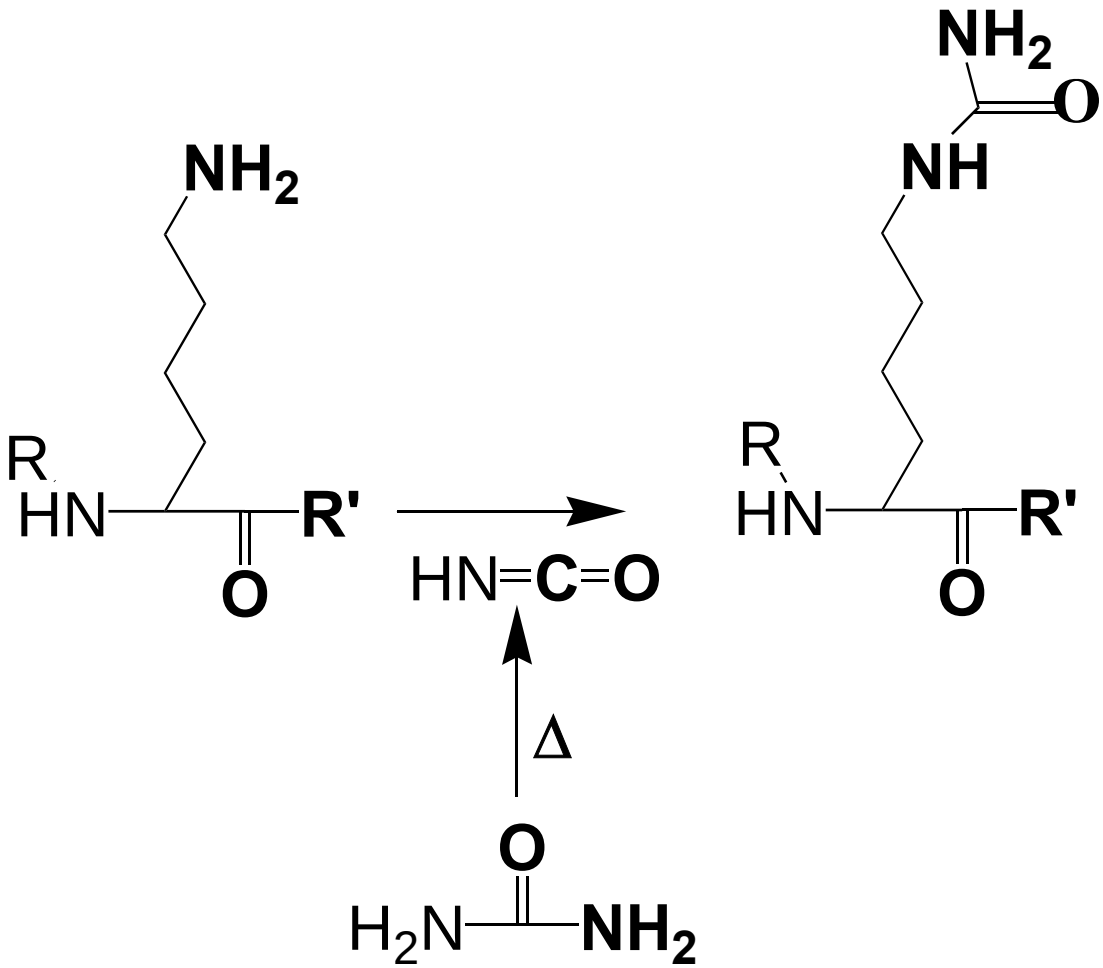
Carbamylation Δ43 Mass

If you use urea,
you mostly like have carbamylations!



<u>S_N2 RxN with Nucleophilic Amino Acids</u>

-N-Term Amine
-Lysine
-Arginine
-Cysteine

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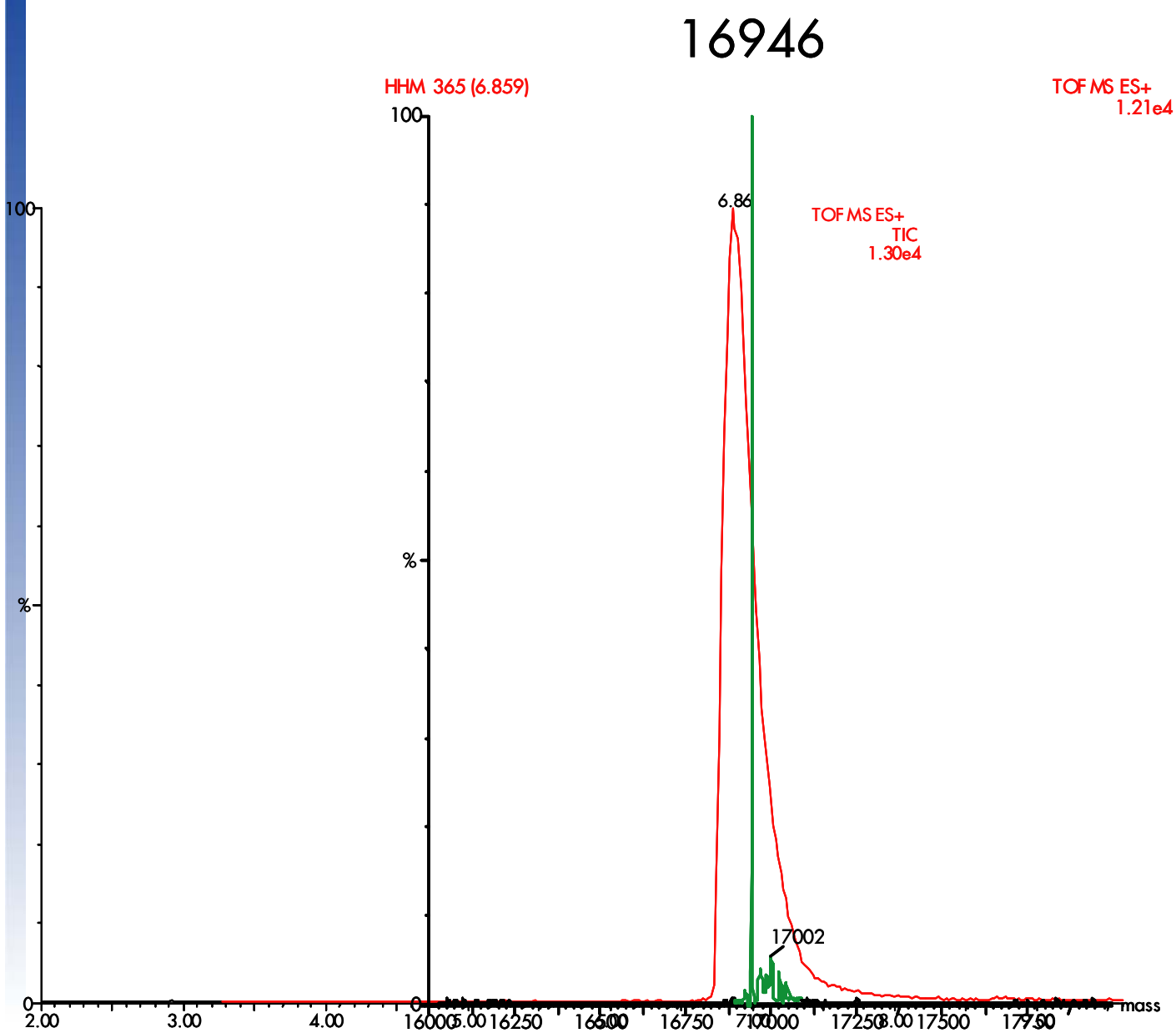
Horse Heart Myoglobin



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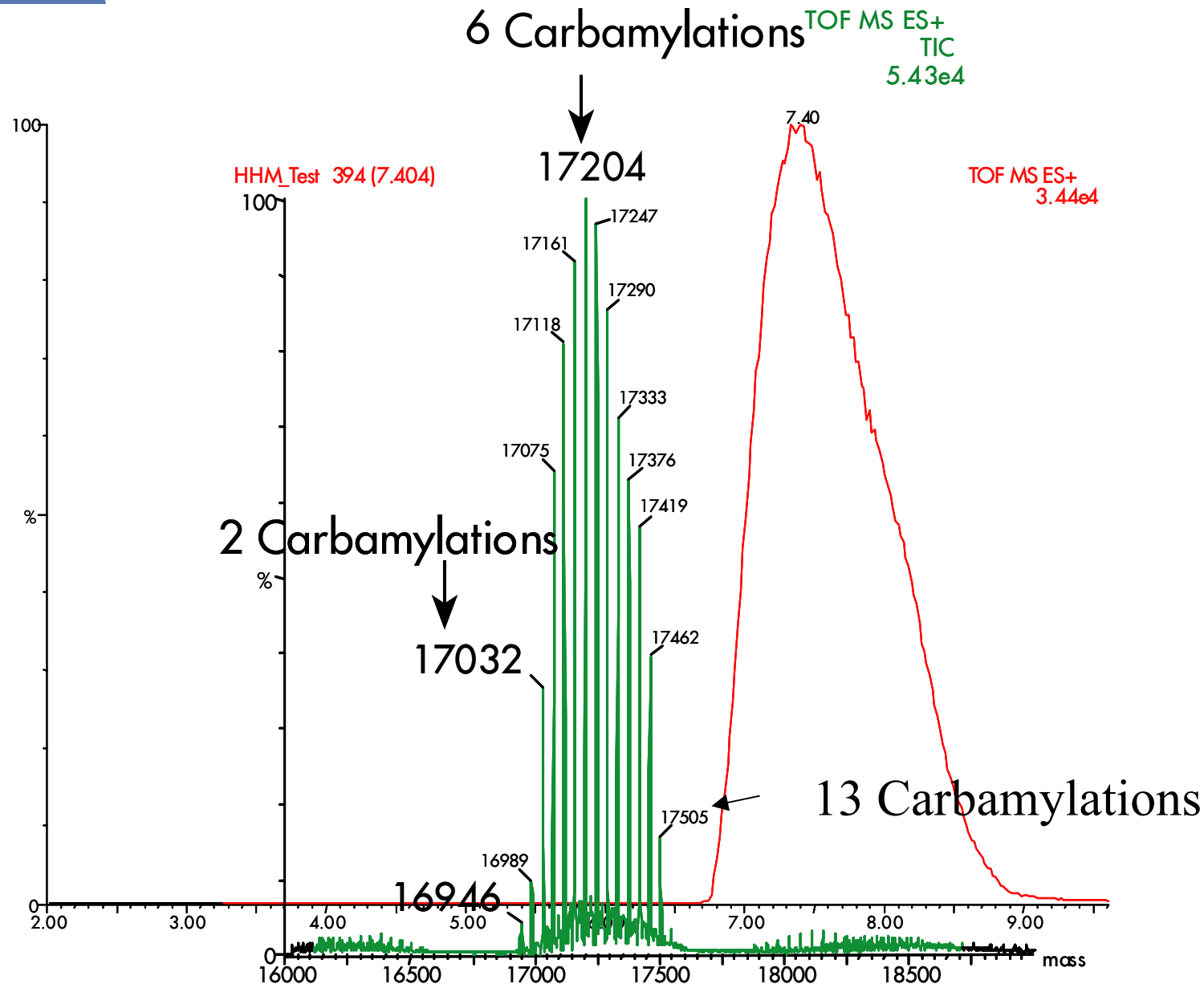
Horse Heart Myoglobin Carbamylated



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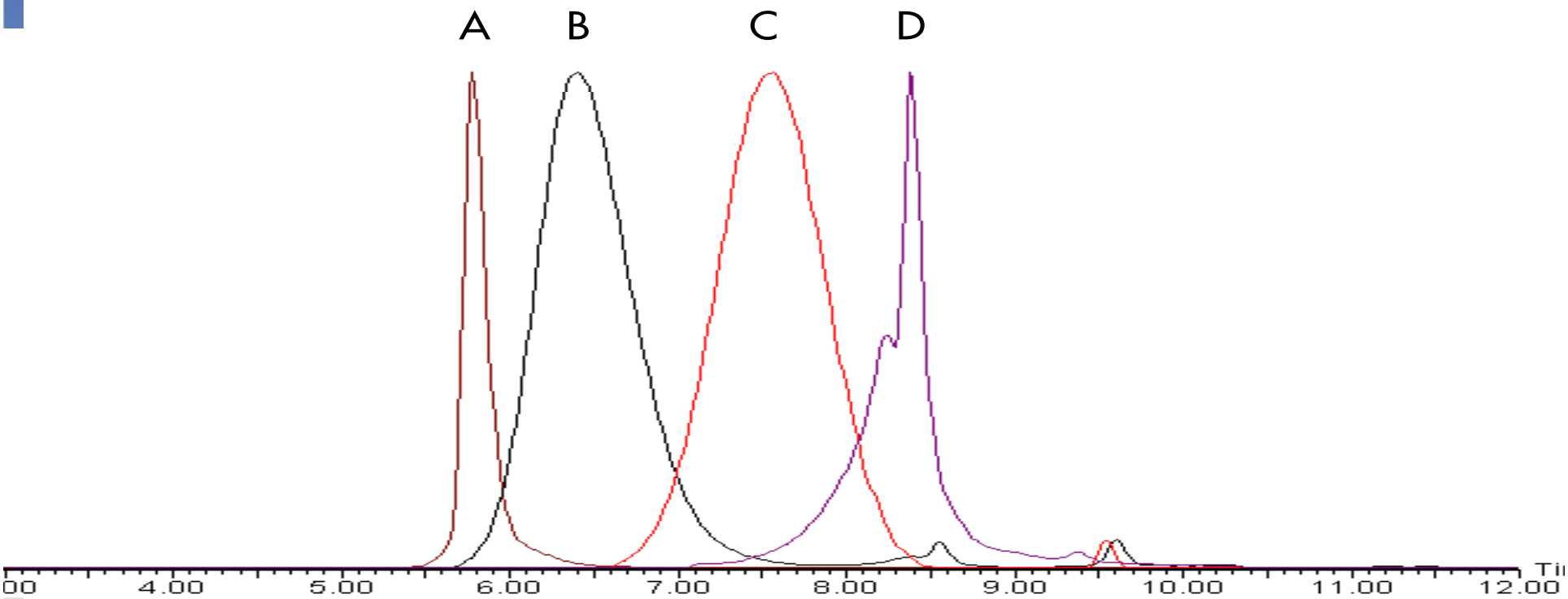
Overlays of LC/MS of Carbamylated Cyto C



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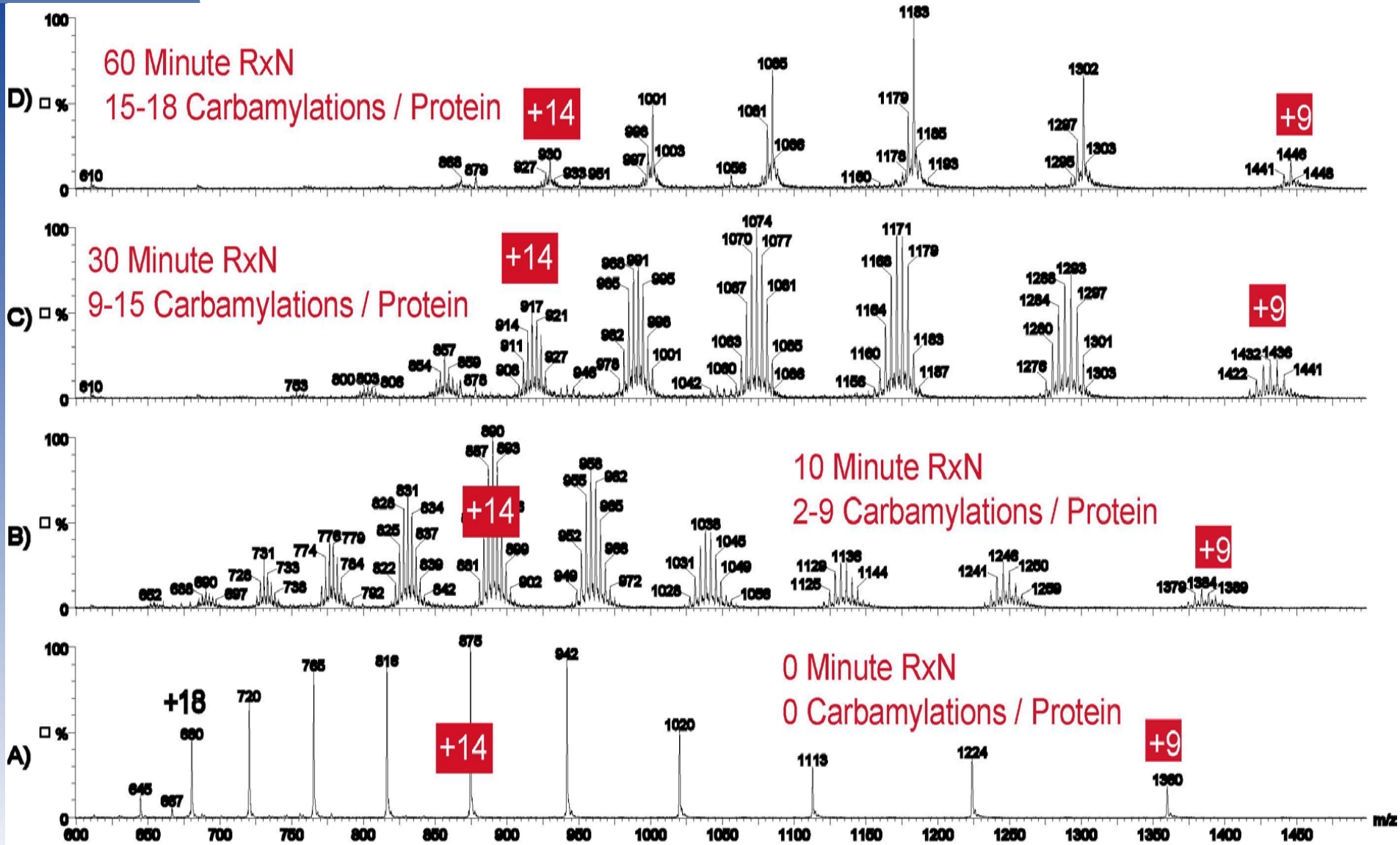
Varying Amounts of Carbamylation



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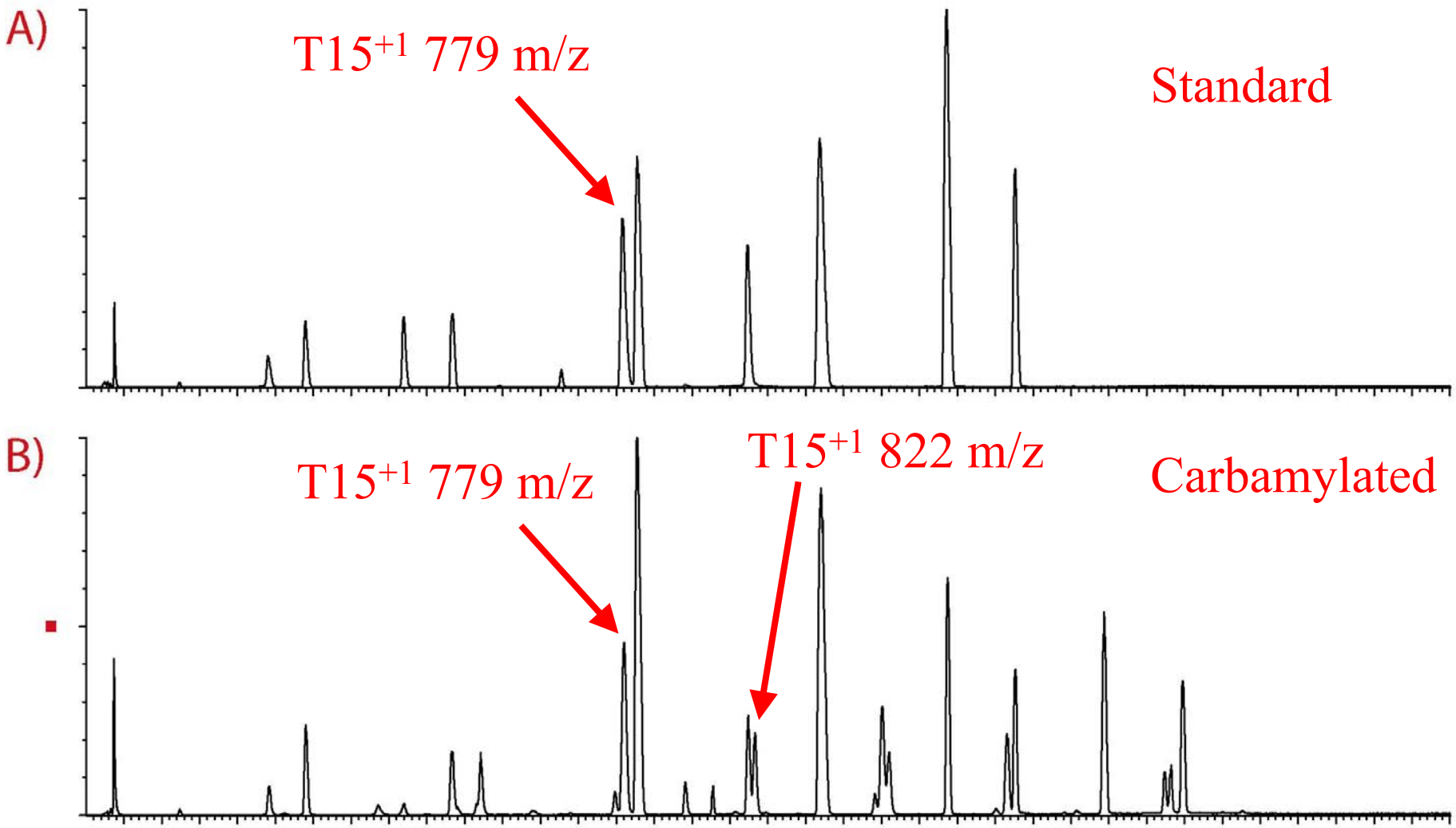
Cyto C Peptides LC/MS



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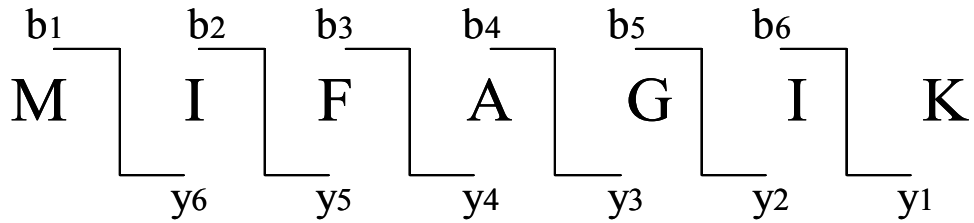
MSMS Ion Comparison T15 (Carbamylation)

Carbamylated N-Term M. →



Not C-Term K

y Ion Series				b Ion Series			
Ion	**m/z Unmodified**	**Δ m/z**	**m/z modified**	**Ion**	**m/z Unmodified**	**Δ m/z**	**m/z modified**
y1	**147**	**Δ 0**	**147**	**b1**	**-**	**-**	**-**
y2	**260**	**Δ 0**	**260**	**b2**	**287**	**Δ 43**	**245**
y3	**317**	**Δ 0**	**317**	**b3**	**435**	**Δ 43**	**392**
y4	**388**	**Δ 0**	**388**	**b4**	**506**	**Δ 43**	**-**
y5	**535**	**Δ 0**	**535**	**b5**	**563**	**Δ 43**	**520**
y6	**648**	**Δ 0**	**648**	**b6**	**-**	**-**	**633**
yMax	**822**	**Δ 43**	**779**	**bMax**	**-**	**-**	**-**

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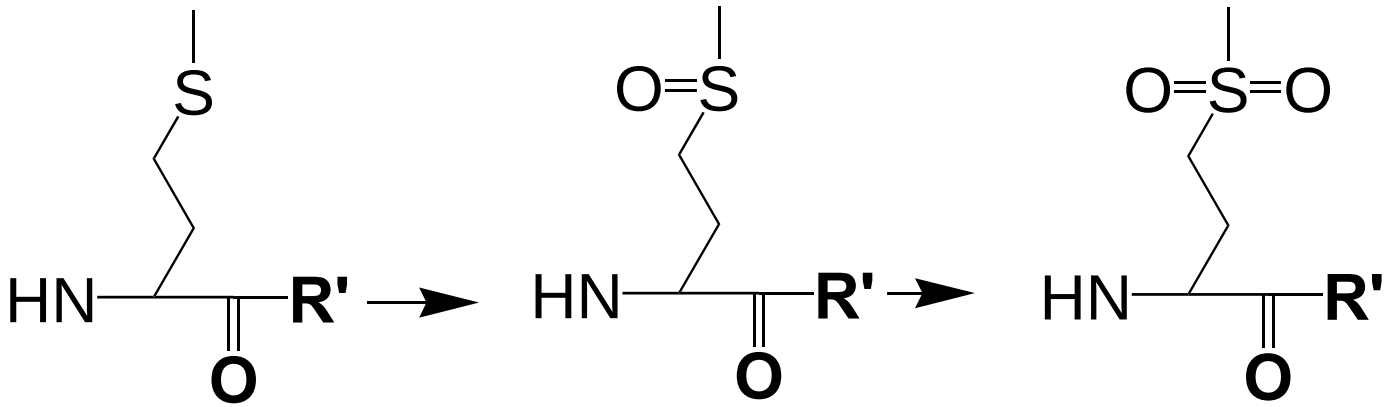
Met Oxidation Δ16 & 32 Mass



Met

Sulfoxide
16 mass

Sulfone
32 mass

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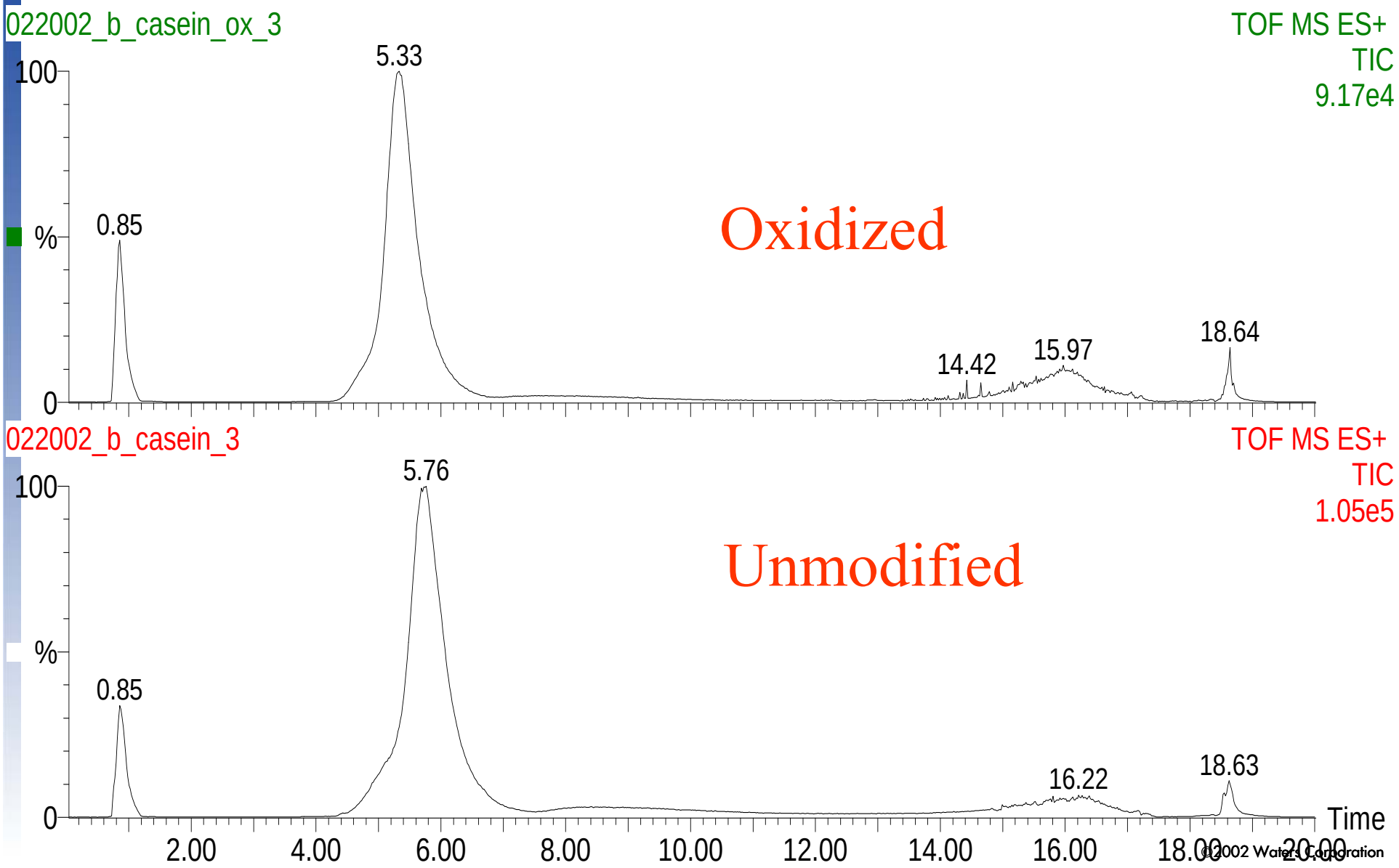
Retention Time Shift for Oxidized β Casein



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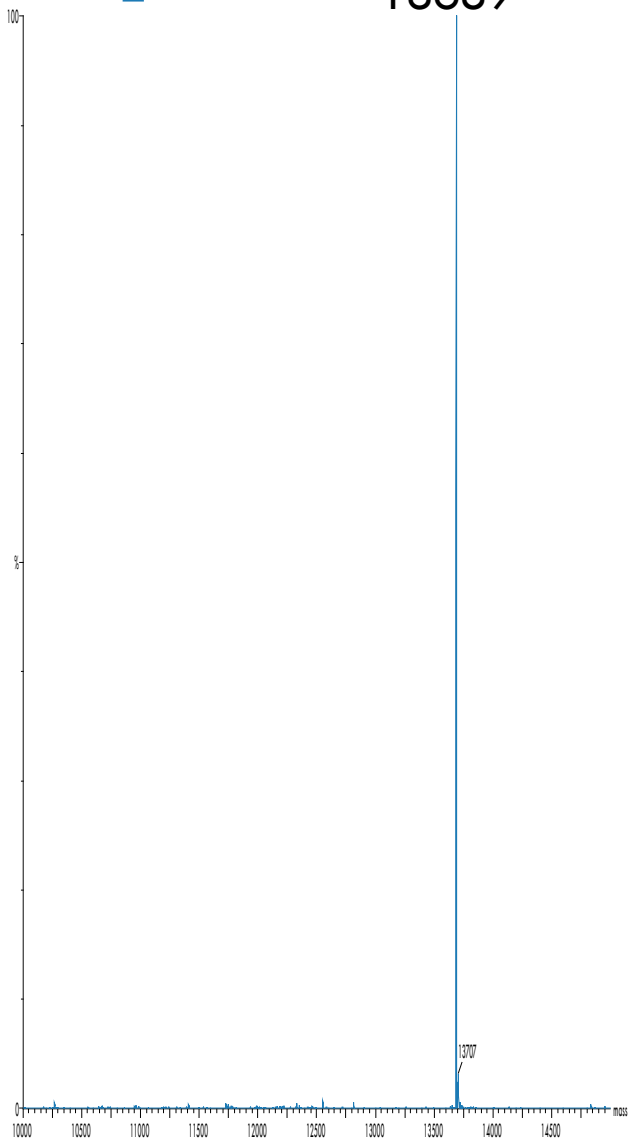
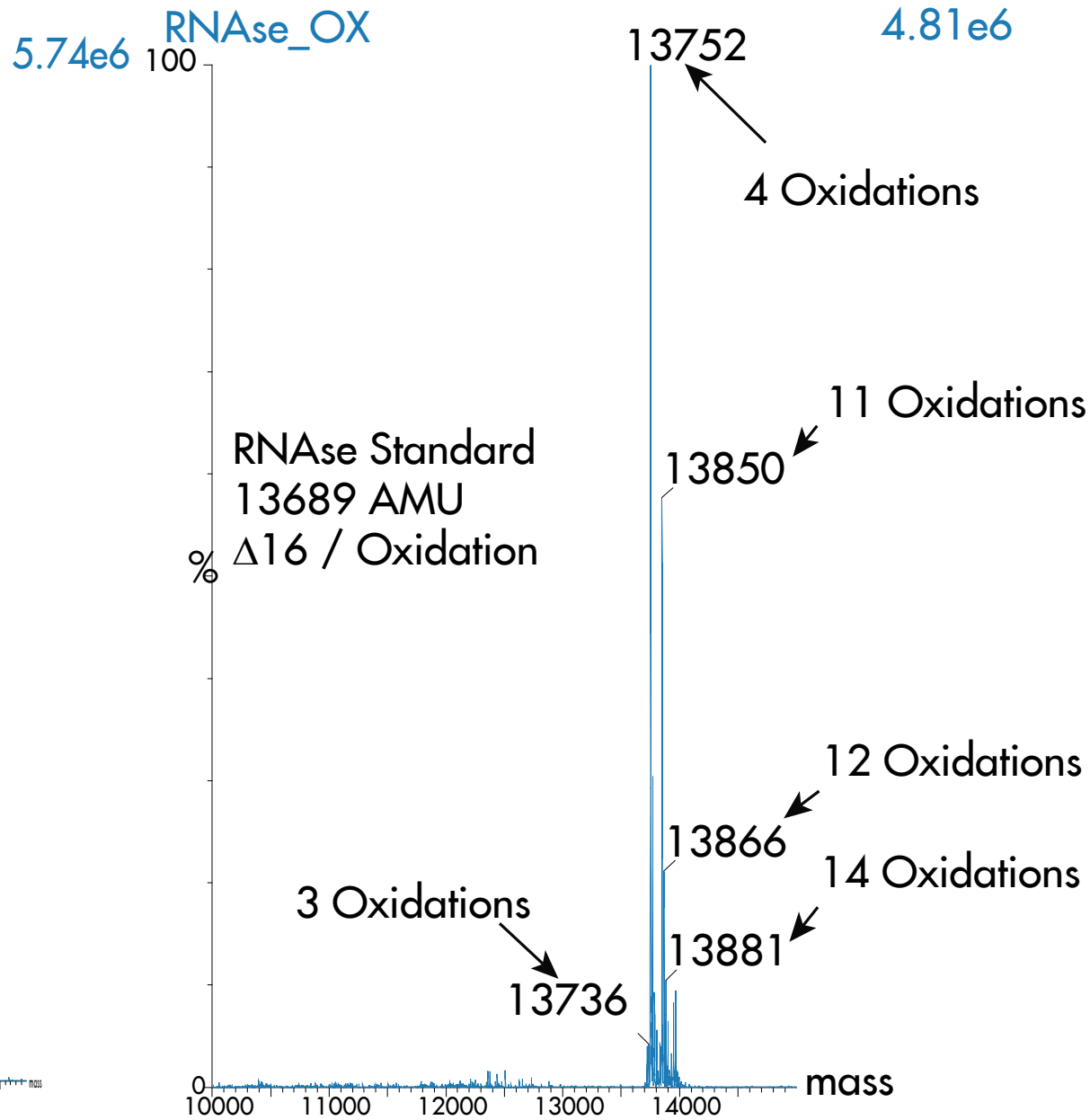
RNAse Protein MS Standard and Oxidized





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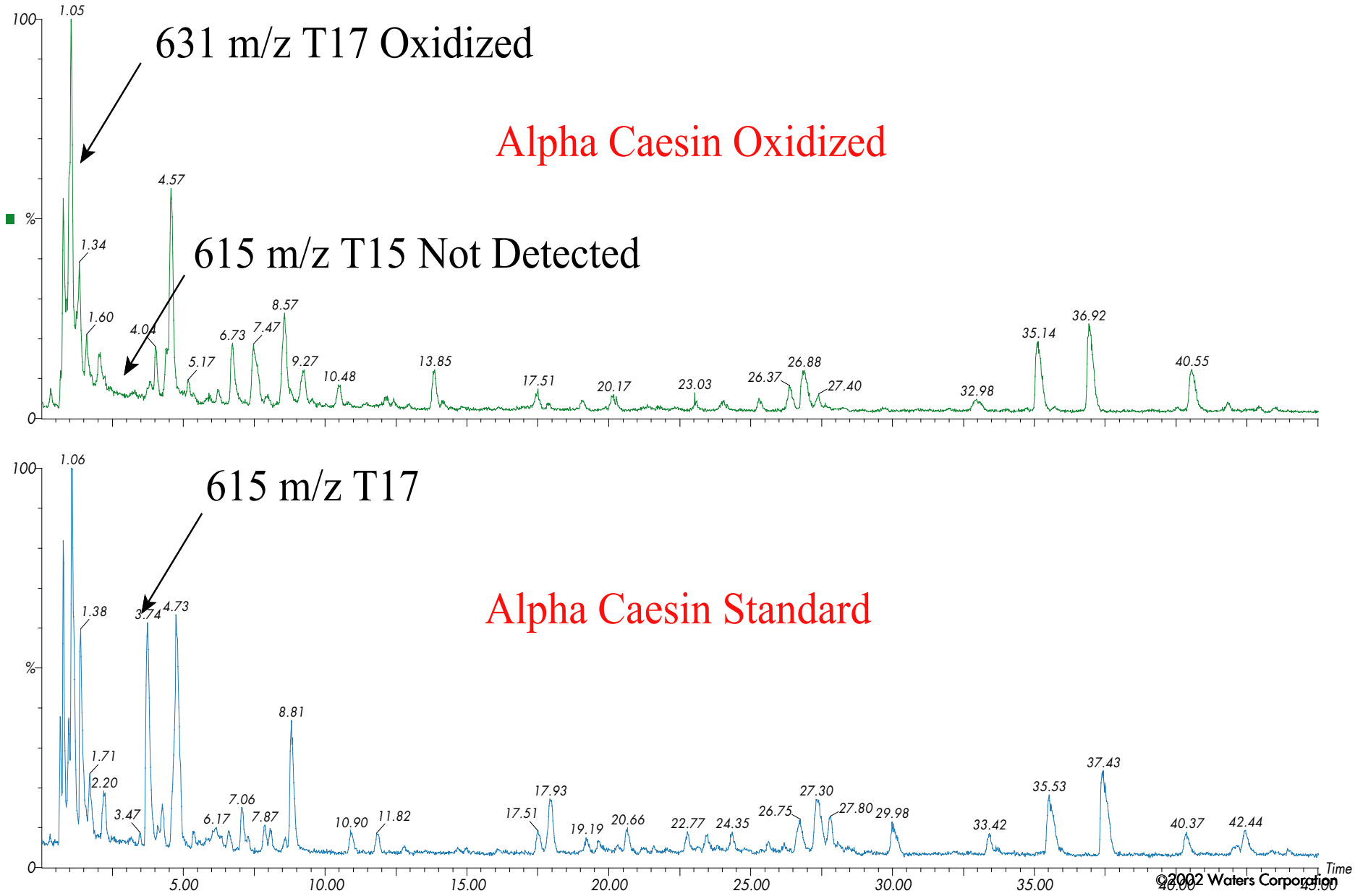
LC/MS: Peptide Unmodified and Oxidized



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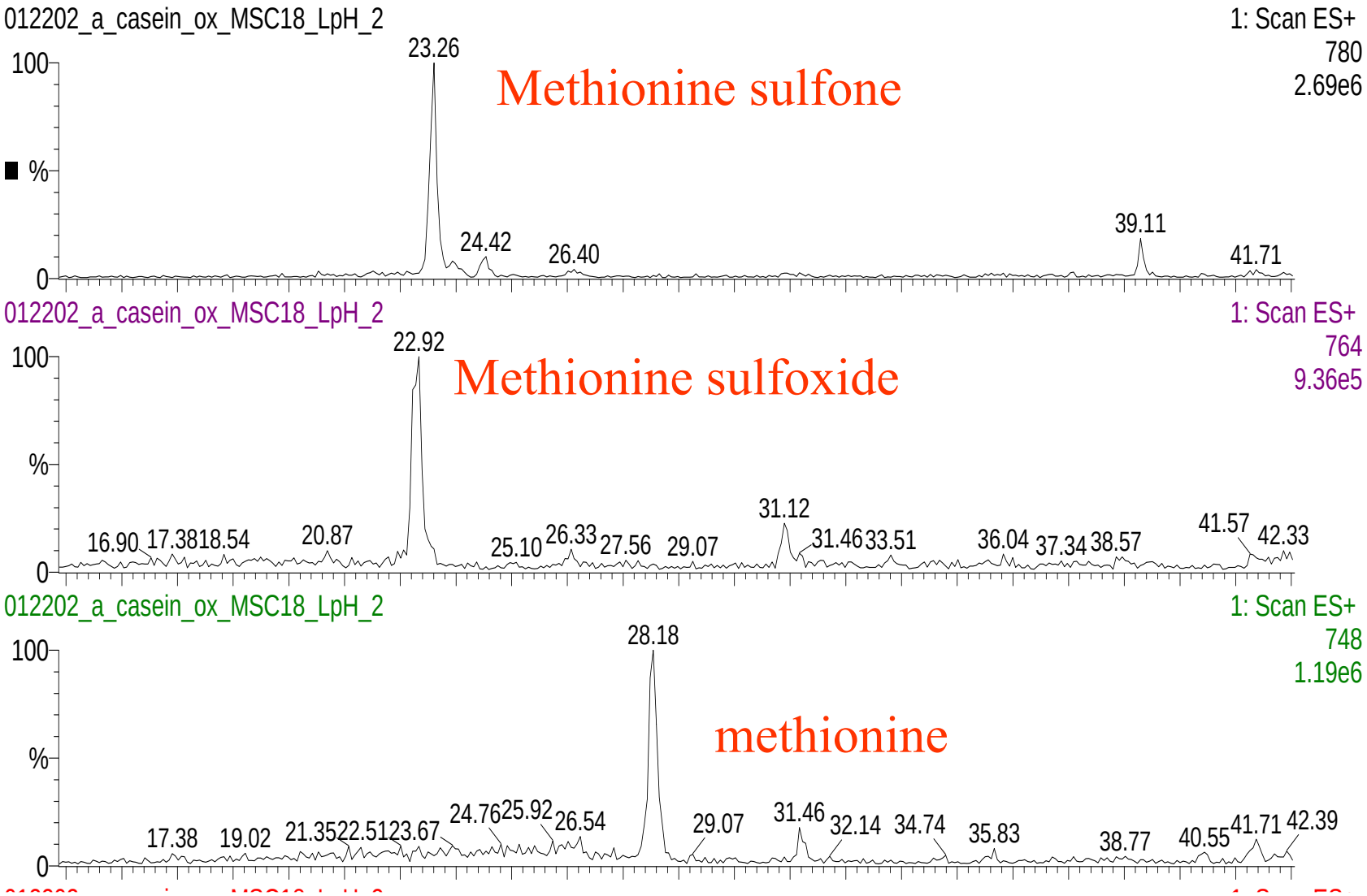
Separation of Oxidized Methionine



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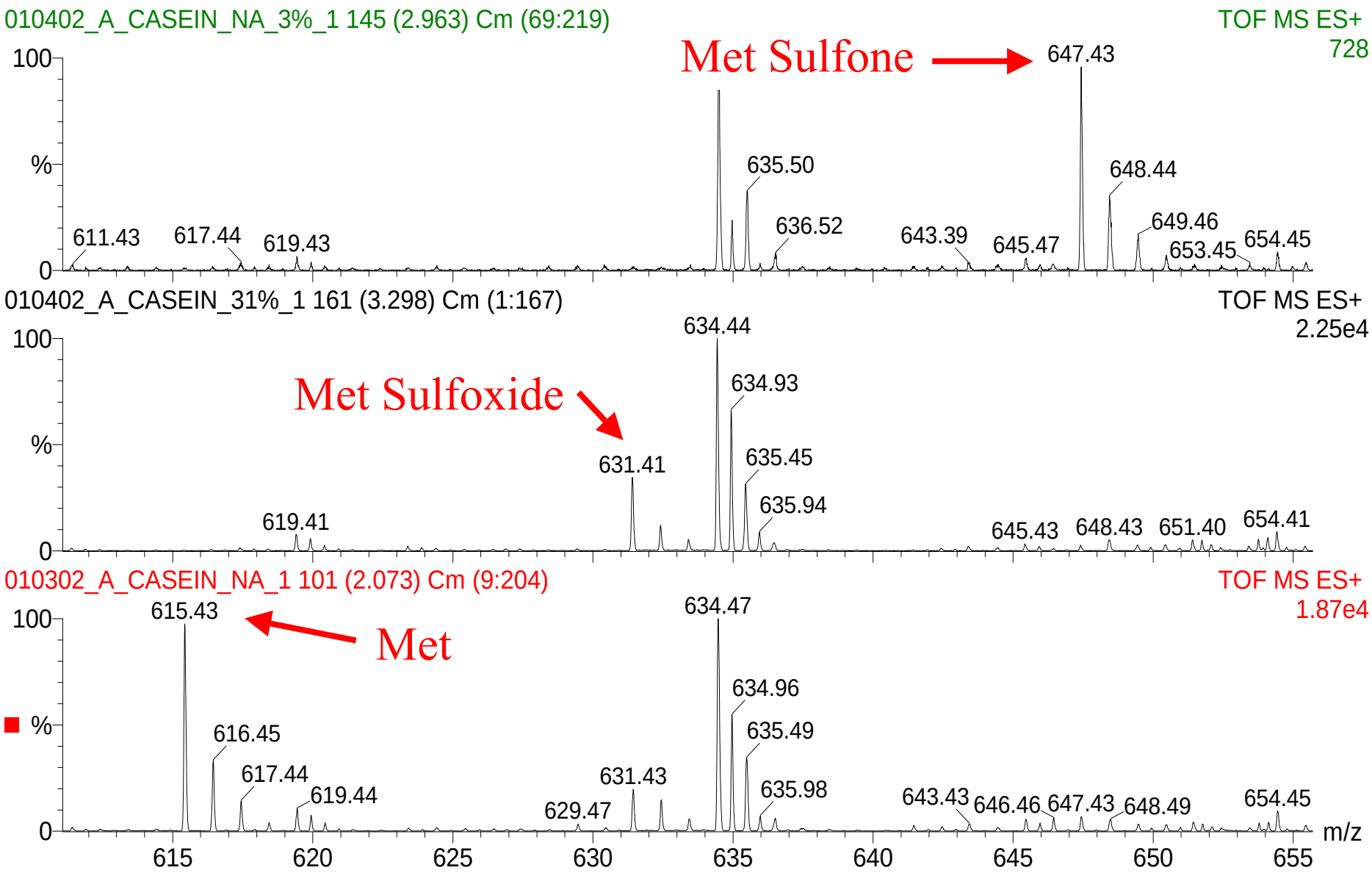
Met Sulfoxide and Sulphone



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MSMS Ion Comparison T17 (Oxidation)

L H S M K

Alpha Casein T17 Fragment

b ion Series				*y ion Series*			
Ion	*m/z standard*	*Δ m/z*	*m/z Ox.*	*Ion*	*m/z standard*	*Δ m/z*	*m/z Ox.*
b1	-	-	-	*y1*	*147*	*Δ0*	*147*
b2	*251*	*Δ0*	*251*	*y2*	*278*	*Δ16*	*294*
b3	*338*	*Δ0*	*338*	*y3*	*365*	*Δ16*	*381*
b4	*467*	-	-	*y4*	*502*	-	-
bmax	*597*	*Δ16*	*613*	*ymax*	*615*	*Δ16*	*631*

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MSMS Ion Comparison T21 (Oxidation)

T T M P L W

Alpha Casein T21 Fragment

b ion Series				*y ion Series*			
Ion	*m/z standard*	*Δ m/z*	*m/z Ox.*	*Ion*	*m/z standard*	*Δ m/z*	*m/z Ox.*
b1	*102*	*Δ0*	*102*	*y1*	*205*	*Δ0*	*205*
b2	*203*	*Δ0*	*203*	*y2*	*318*	*Δ0*	*318*
b3	*334*	*Δ16*	*350*	*y3*	*415*	*Δ0*	*415*
b4	*431*	*Δ16*	*447*	*y4*	*546*	*Δ16*	*562*
b5	*544*	*Δ16*	*560*	*y5*	-	-	-
bmax	*730*	*Δ16*	*746*	*ymax*	*748.5*	*Δ16*	*764.5*

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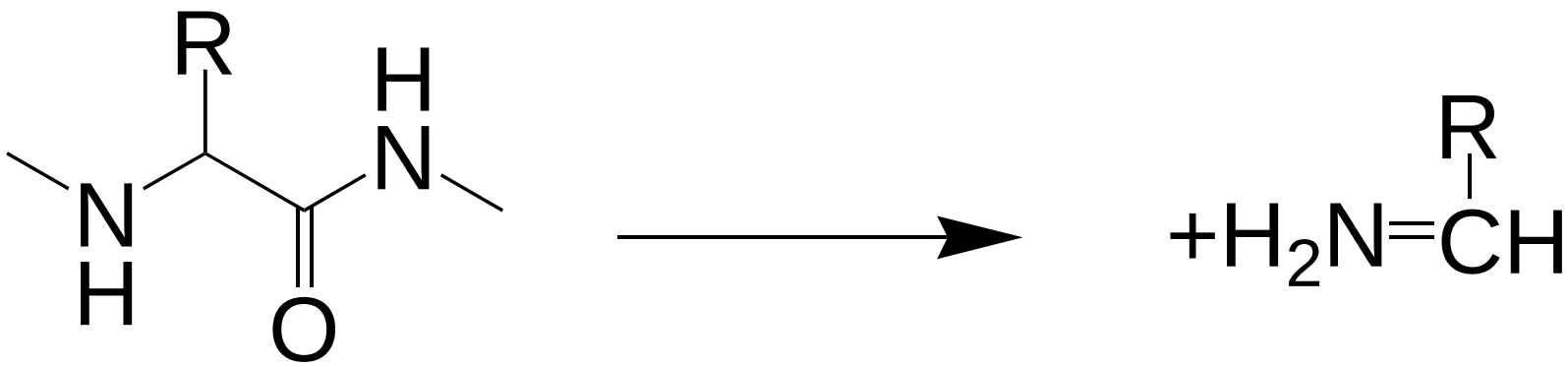
Immonium Ions



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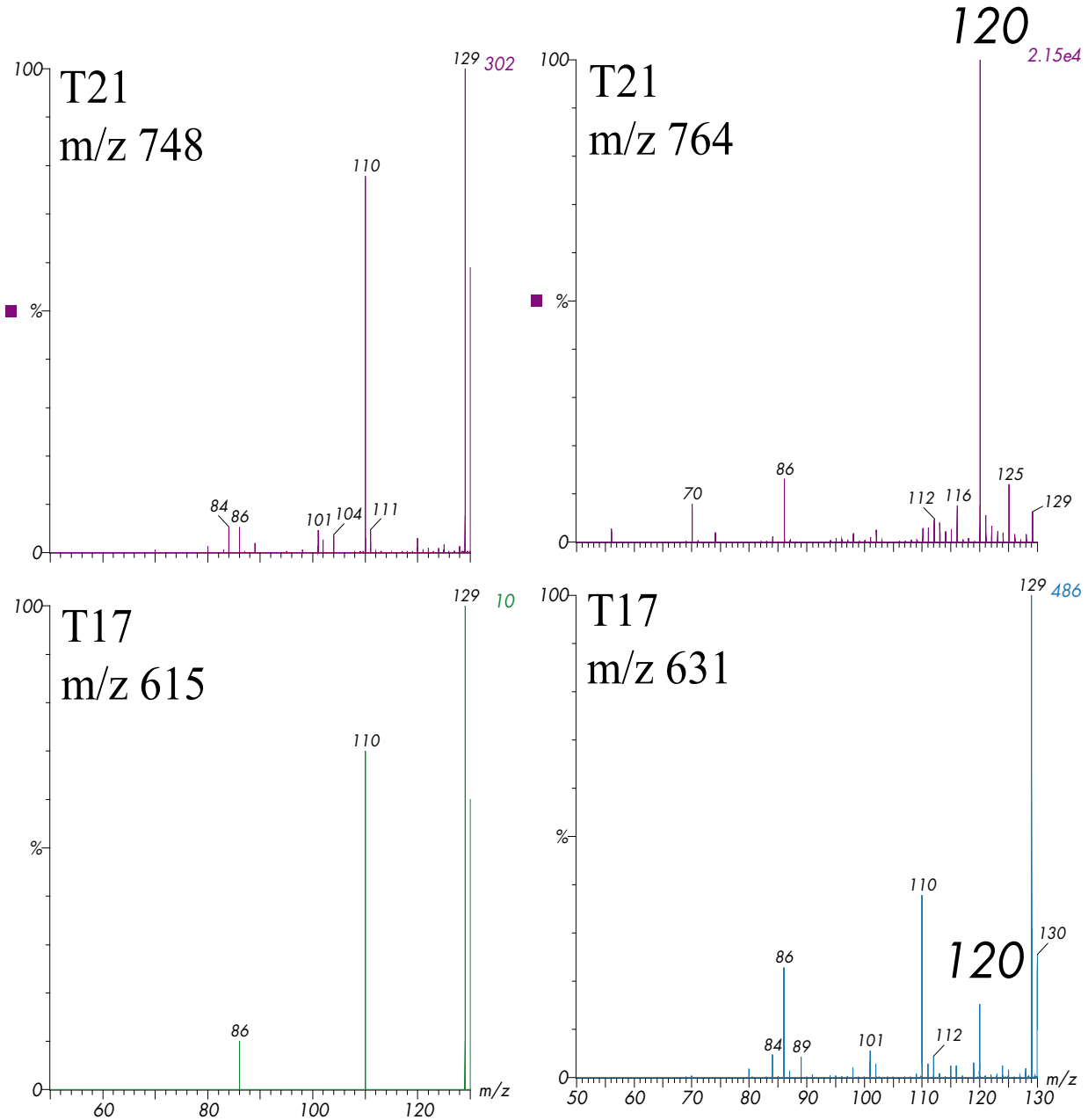
Immonium Ions Indicative of Met^{Ox}



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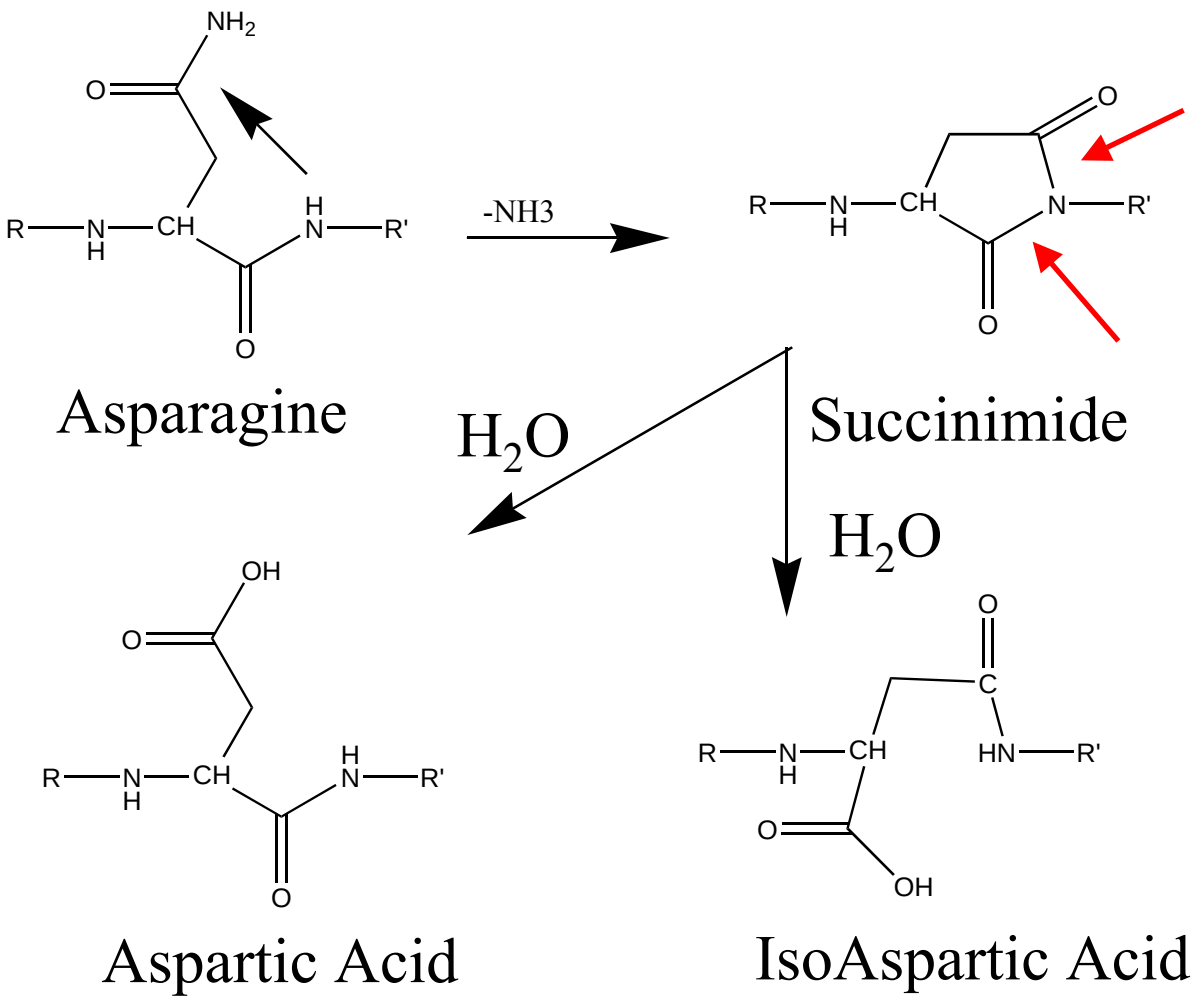
Asp Deamidation Δ1 m/z



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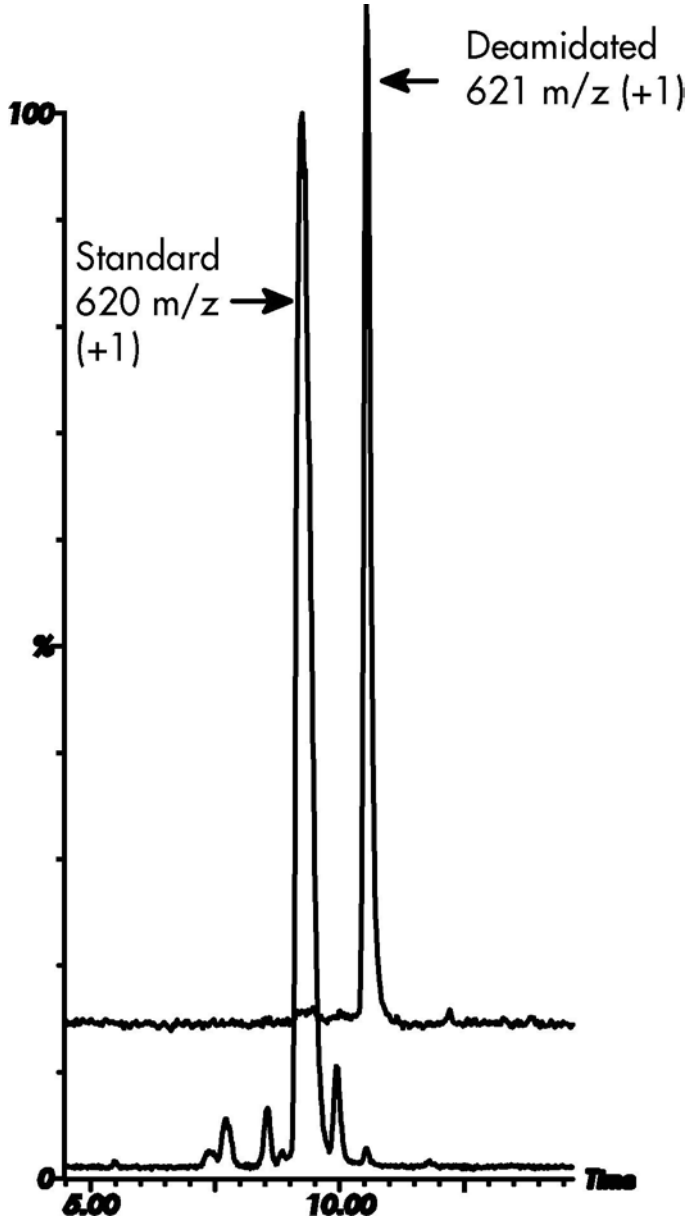
Retention Shift: Deamidated ACTH Peptides



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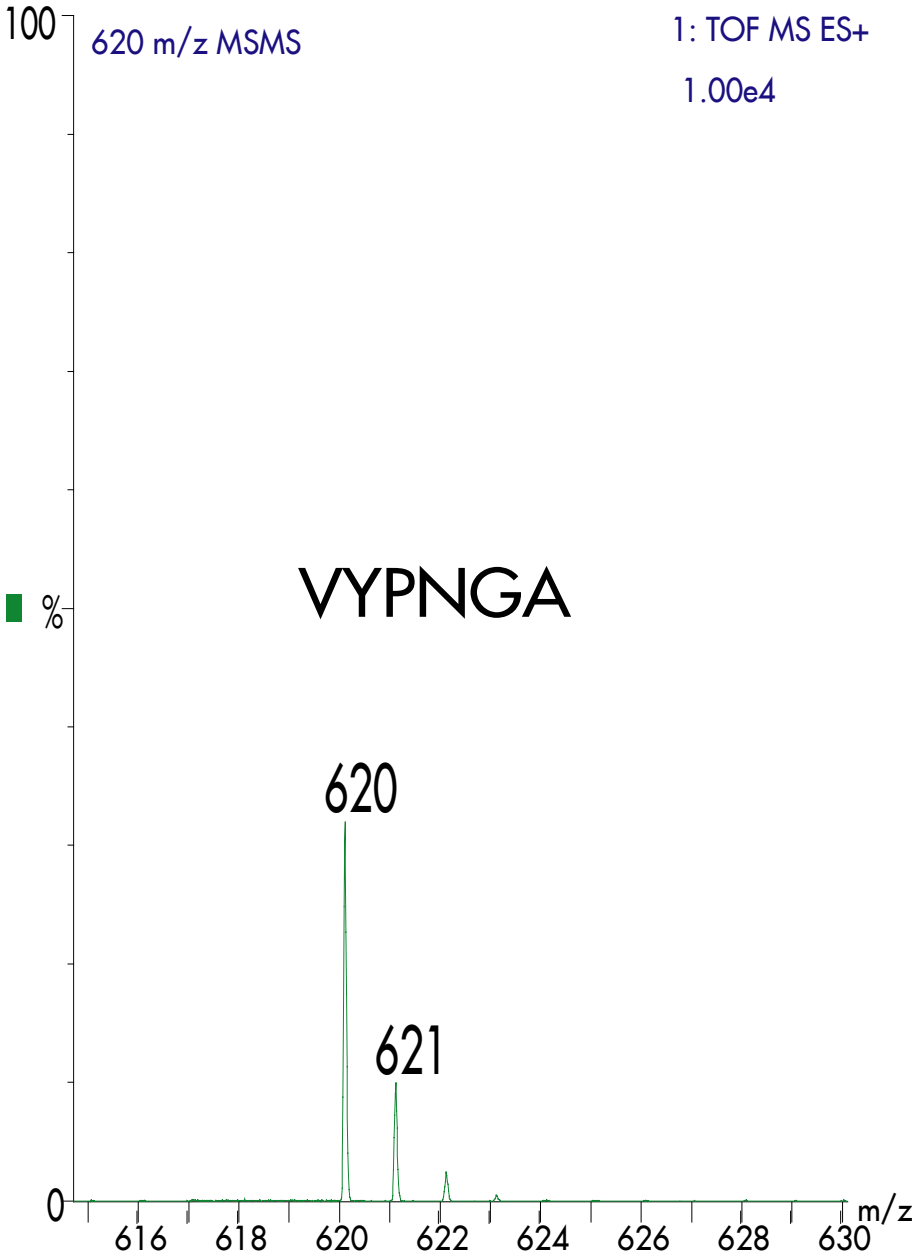
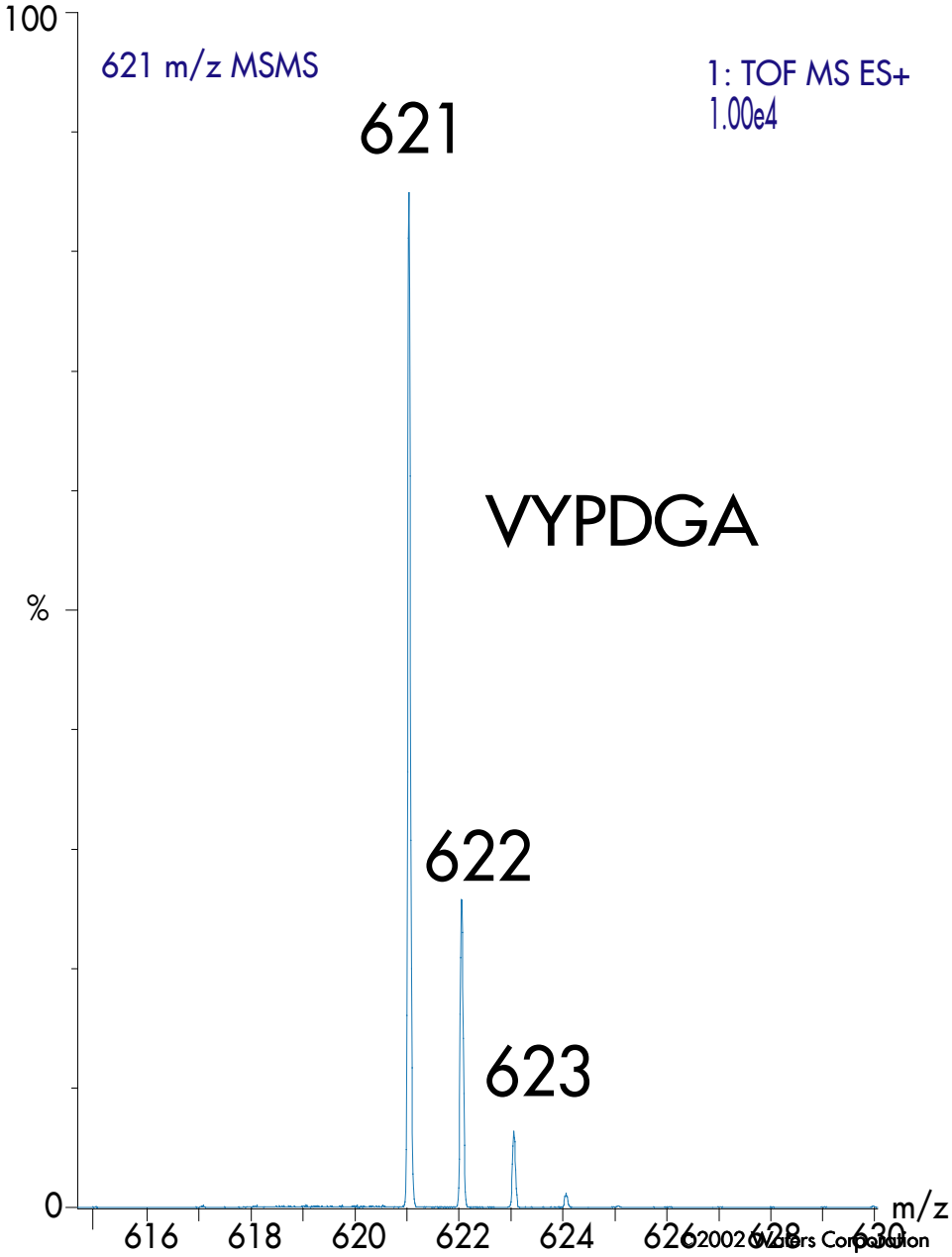
Isotope Patterns of Deamidation





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MSMS Ion Comparison ACTH Peptides

V Y P N/D G A

ACTH Peptide

b ion Series				y ion Series			
Ion	m/z standard	Δ m/z	m/z Ox.	Ion	m/z stan-dard	Δ m/z	m/z Ox.
b1	-	-	-	y1	-	Δ0	-
b2	263	Δ0	263	y2	147	Δ0	147
b3	-	-	360	y3	261	Δ1	262
b4	-	-	-	y4	258	Δ1	359
b5	-	-	-	y5	521	Δ1	522
b_{max}	-	-	-	y_{max}	620	Δ1	621

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Summary

A Simple, Universal Methodological Approach for the Characterization of

- *Carbamylation, Oxidation, Deamidation*

- *LC/UV Proteins*
- *LC/MS Proteins*

- *LC/UV Peptides from Proteins*
- *LC/MS Peptides from Proteins*
- *LC/MS/MS Peptides from Proteins*

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Modification Effects

Sample	Retention Time	Δ Change	Signature MSMS ions
Carbamylation			
Peptide	Increase	43	144,172,119*
Protein	Increase	43	
Oxidation			
Peptide	Decrease	16/32	120 Actual
Protein		16	
Deamidation			
Peptide	Increase	1	88*

*Theoretical

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In Progress

Same methods described here we have done or are doing for;

- *Phosphorylation*
- *Formylation*
- *Acylation*

Future

- *Glycosylation*

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ACS Poster; Wednesday 6-9

- *248 Identification and Characterization of Post Translational Modifications of Proteins by LC/MS*
- *249 Detecting Carbamylation of Intact Proteins by LC/MS*
- *265 Evaluation of Reversed Phase Columns for Protein Separation using a Single LC/UV/MS Methodology*
- *284 Significantly improved in-solution tryptic digestion of proteins*

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