CHARACTERIZATION OF PROTEIN MODIFICATIONS USING LIQUID CHROMATOGRAPHY AND DATA INDEPENDENT ACQUISITION TANDEM MASS SPECTROMETRY

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INTRODUCTION

Covalent modifications such as oxidation and deamidation are common in recombinant protein products. They have the potential to affect the safety, activity and stability of protein drugs. Sensitive methods for effective monitoring of such modifications and quality control are required.

We have applied Ultra Performance Liquid Chromatography–Data Independent Acquisition Tandem Mass Spectrometry (UPLC-MS^E) approach for analysis of a tryptic digest from the monoclonal antibody Herceptin. Deamidation and methionine (M) oxidation were identified and quantified. Synthetic peptides were used to help distinguish M-oxidation in the protein from in-source M-oxidation, and modified isoforms of deamidated peptides, specifically the "PENNY" peptide.

ADVANTAGES OF UPLC-MS^E

ACQUITY UPLC®

- **Improved peptide resolution**
- **Improved detection sensitivity**
- **Improved speed and efficiency**

MS^E

- **Acquiring precursor and fragment ions in parallel**
- **Data independent acquisition (DIA)**
- **Unbiased sampling of low-abundance peptides**

Elution Order of Synthetic Peptides

Synthetic Peptides[1]	RT (min)	Elution Order
A)		
GFYPSDIAVEWESNGQPENNYK	59.76	3
GFYPSDIAVEWESisoDGQPENNYK	N/A[2]	1
GFYPSDIAVEWESDGQPENNYK	60.57	7
GFYPSDIAVEWESNGQPEisoDNYK	59.66	2
GFYPSDIAVEWESNGQPEDNYK	60.31	5
GFYPSDIAVEWESNGQPENDYK	60.14	4
GFYPSDIAVEWESNGEPENNYK	60.31	5
GFYPSDIAVEWESDGQPEDNYK	61.06	8
GFYPSDIAVEWESDGQPEDDYK	61.55	9
B)		
DIQMTQSPSSLSASVGDR	42.85	2
DIQMoxTQSPSSLSASVGDR	36.07	1

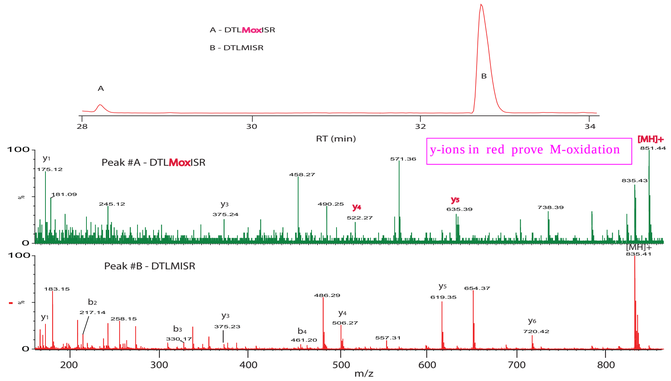
[1] *A) N/Q-deamidation, "PENNY" peptide T37 of heavy chain; B) M-oxidation, T1 of light chain;*
isoD - isoaspartic acid; Mox - oxidized M.
[2] N/A - not available.

Modification Type, Site and Stoichiometry of Modified Peptides Identified from Herceptin Antibody

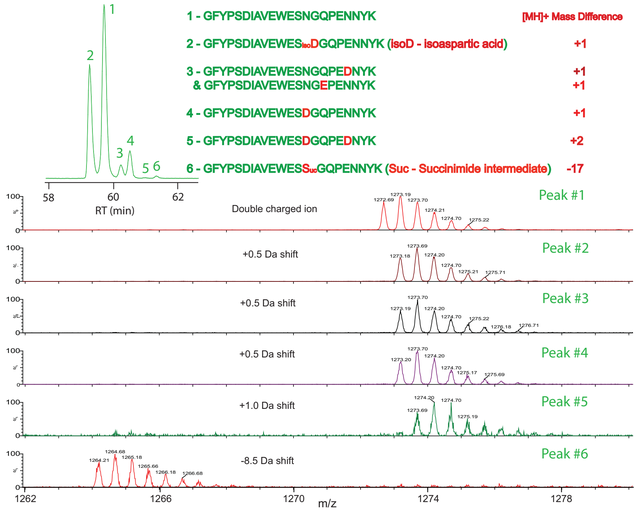
Protein	Peptide	Start	End	Modification Type	Sequence[1] & Modification Site	MH+	RT (min)	SC (%)[2]
Heavy-Chain	T6	51	59	Deamidation N55	IYPTNGYTR	1085.53	27.69	5.5
	T6	51	59	Deamidation N55	IYPTNGYTR	1085.53	28.8	46.2
	T6	51	59	No Modification	IYPTNGYTR	1084.55	26.96	48.3
	T10	77	87	Deamidation N84	NTAYLQMNSLR	1311.64	42.47	65.1
	T10	77	87	Deamidation N84	NTAYLQMNSLR	1311.64	45.36	13.4
	T10	77	87	No Modification	NTAYLQMNSLR	1310.66	43.7	21.5
	T23	278	291	Deamidation N289	FNWYVDGVEVHNAK	1678.79	51.13	5.1
	T23	278	291	Deamidation N289	FNWYVDGVEVHNAK	1678.79	51.78	9.3
	T23	278	291	No Modification	FNWYVDGVEVHNAK	1677.81	50.07	85.6
	T36	364	373	Deamidation N364	NQVSLTCLVK	1162.61	42.85	1.6
	T36	364	373	Deamidation N364	NQVSLTCLVK	1162.61	49.12	2.1
	T36	364	373	No Modification	NQVSLTCLVK	1161.63	47.06	96.3
	T37	374	395	Deamidation N387	GFYPSDIAVEWESNGQPENNYK	2545.12	59.36	39.2
	T37	374	395	Deamidation N387	GFYPSDIAVEWESNGQPENNYK	2545.12	60.57	9.4
	T37	374	395	Deamidation N392 & Deamidation N389	GFYPSDIAVEWESNGQPENNYK & GFYPSDIAVEWESNGQPENNYK	2545.12	60.31	3.2
	T37	374	395	Deamidation N387 + N392	GFYPSDIAVEWESNGQPENNYK	2546.1	61.06	0.4
	T37	374	395	Succinimide Intermidate N387	GFYPSDIAVEWESNGQPENNYK	2527.1	61.38	1.3
	T37	374	395	No Modification	GFYPSDIAVEWESNGQPENNYK	2544.14	59.76	46.4
	T21	252	258	Oxidation M255	DTLMISR	851.43	28.23	4.7
	T21	252	258	No Modification	DTLMISR	835.43	32.76	95.3
Light-Chain	T3	25	42	Deamidation N30	ASQDVNTAVAWYQQKPGK	1291.98	38.6	1.9
	T3	25	42	Deamidation N30	ASQDVNTAVAWYQQKPGK	1291.98	41.69	32.6
	T3	25	42	No Modification	ASQDVNTAVAWYQQKPGK	1291	40.34	65.5
	T11	127	142	Deamidation N137	SGTASVVCLLNNFYPR	1798.88	62.05	6.3
	T11	127	142	Deamidation N137	SGTASVVCLLNNFYPR	1798.88	74.61	3.3
	T11	127	142	No Modification	SGTASVVCLLNNFYPR	1797.9	72.05	90.4
	T14	150	169	Deamidation N158	VDNALQSGNSQESVTEQDSK	2136.95	26.88	3.8
	T14	150	169	Deamidation N158	VDNALQSGNSQESVTEQDSK	2136.95	28.11	3.2
	T14	150	169	No Modification	VDNALQSGNSQESVTEQDSK	2135.97	27.1	93

[1] C* - carbamidomethyl C
[2] Stoichiometry in percentage, detected in freshly prepared sample

Elution Pattern and MS^E Spectra of Peptide T21 in Heavy-Chain Before and After M-oxidation

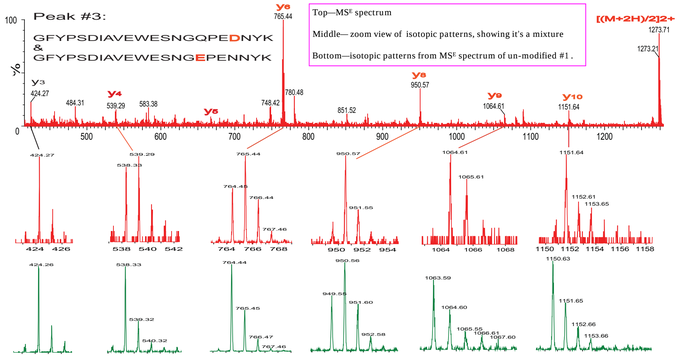


Elution Pattern and MS Spectra of Peptide T37 in Heavy-Chain Before and After Deamidation

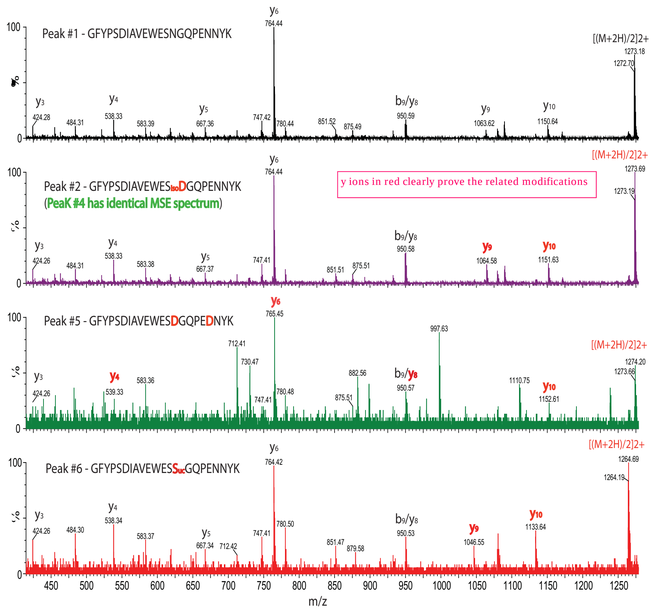


MS^E Spectrum of Peak #3 and Isotopic Patterns of y-series Ions

(Confirming the Co-elution of 2 Modified "PENNY" Peptides)



MS^E Spectra of Peaks # 1, #2 /#4, #5, and #6



CONCLUSIONS

1. **Peptide mapping with UPLC-MS^E is an approach with high resolution, high sensitivity, unbiased identification. It's capable of separating, identifying and quantifying modifications in the antibody with stoichiometry as low as 0.5%; as well as providing high sequence coverage (97% for both light and heavy chains).**
2. **The approach offers increased speed of analysis.**
3. **The high mass resolution and high mass accuracy of SYNAPT HDMS™ system ensures confident identification of modifications with small mass shift (e.g., N-deamidation with 0.98 Da mass difference) and modified isoforms.**
4. **Synthetic peptides are helpful for determining modified isoforms.**
5. **In summary, UPLC-MS^E and SYNAPT HDMS™ system is an advanced platform for characterization of recombinant proteins, such as monoclonal antibodies.**

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