Characterization of a Monoclonal Antibody Using Peptide Mapping with UPLC/MS^E

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SUMMARY

Covalent modifications such as oxidation and deamidation are common in monoclonal antibodies. The modified motif potentially affects the safety, activity and stability of these drugs. Sensitive methods for effective monitoring of such modifications are required.

We have applied an Ultra Performance Liquid Chromatography–Data Independent Acquisition Mass Spectrometry (UPLC/MS^E) approach for analysis of a tryptic digest from a therapeutic antibody. High sequence coverage (>97%) was reproducibly obtained. Site-specific deamidation and methionine (M) oxidation were identified and quantified. Synthetic peptides were used to further confirm the deamidations in the "PENNY" peptide of heavy chain, distinguish aspartyl and isoaspartyl isoforms of N-deamidations, and exclude potential M-oxidation artifact produced in the MS ion source.

ADVANTAGES OF UPLC/MS^E

ACQUITY™ UPLC® System

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

MS^E

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

ELUTION ORDER OF SYNTHETIC PEPTIDES

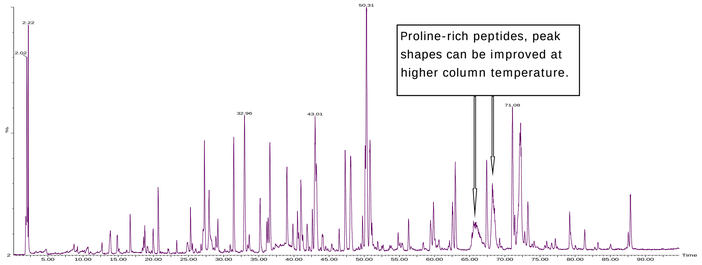
Synthetic Peptides[1]	RT (min)	Elution Order
A)		
GFYPSDIAVEWESNGQPENNYK	59.76	3
GFYPSDIAVEWESisoDGQPENNYK	59.36	1
GFYPSDIAVEWESDGQPENNYK	60.57	7
GFYPSDIAVEWESNGQPEisoDNYK	59.66	2
GFYPSDIAVEWESNGQPEDNYK	60.31	5
GFYPSDIAVEWESNGQPENDYK	60.14	4
GFYPSDIAVEWESNGEPENNYK	60.31	5
GFYPSDIAVEWESDGQPEDNYK	61.06	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.

UPLC/MS CHROMATOGRAM (TIC) OF AN ANTIBODY TRYPTIC DIGEST

The UPLC-eluted Peptides Identified by MS^E cover 97% Sequences of Both Light and Heavy Chains of the Antibody

LC System:	Waters ACQUITY™ UPLC®	Column :	ACQUITY™ PST C18, BEH300Å, 2.1 x 150 mm, 1.7 µm		
Mobile Phase A:	0.1% FA in water	Mobile Phase B:	0.1% FA in acetonitrile	Column Temp:	40 °C
Gradient:	0-50% B in 90 min	Flow Rate:	0.2 ml/min	Detection:	MS^E

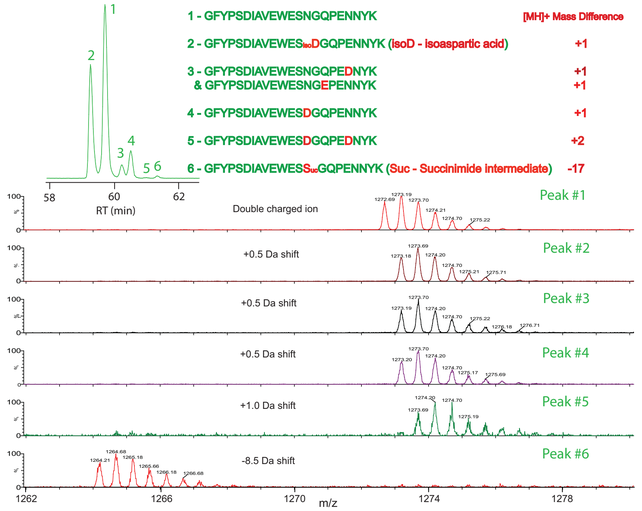


MODIFICATION TYPE, SITE AND STOICHIOMETRY OF MODIFIED PEPTIDES IDENTIFIED FROM THE ANTIBODY

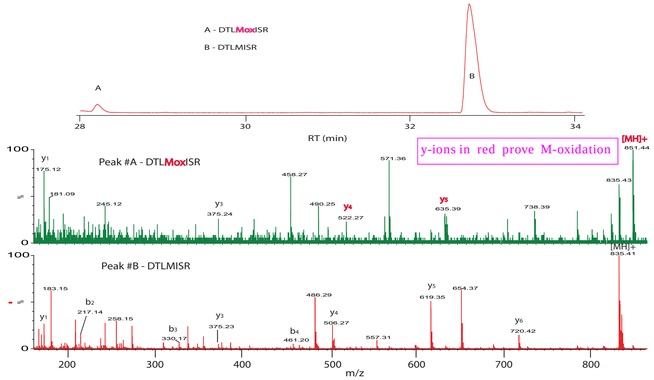
Protein	Peptide	Start	End	Modification Type	Sequence[1] & Modification Site	MH+	RT (min)	SC (%)[2]
Heavy-Chain	T6	51	59	Deamidation N55	IYPTNGYTR	1085.53	27.69	5.5
	T6	51	59	Deamidation N55	IYPTNGYTR	1085.53	28.8	46.2
	T6	51	59	No Modification	IYPTNGYTR	1084.55	26.96	48.3
	T10	77	87	Deamidation N84	NTAYLQMNSLR	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	NQVSLTC*LVK	1162.61	42.85	1.6
	T36	364	373	Deamidation N364	NQVSLTC*LVK	1162.61	49.12	2.1
	T36	364	373	No Modification	NQVSLTC*LVK	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	T1	1	18	Oxidation M4	DIQMTQSPSSLSASVGDR	1894.88	43.41	in-source
	T1	1	18	No Modification	DIQMTQSPSSLSASVGDR	1878.88	43.41	
	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	SGTASVVC*LNNFYPR	1798.88	67.01	2.51
	T11	127	142	Deamidation N137	SGTASVVC*LNNFYPR	1798.88	74.61	3.5
	T11	127	142	No Modification	SGTASVVC*LNNFYPR	1797.9	72.05	93.99
	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] SC - Stoichiometry in percentage, detected in freshly prepared sample

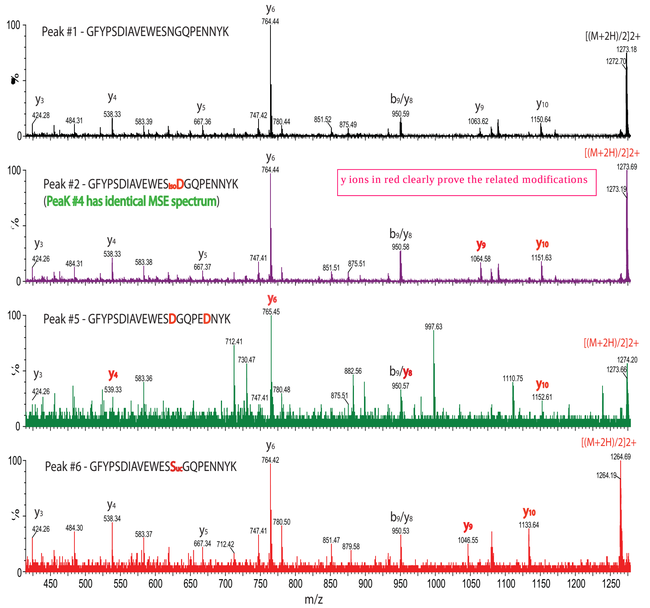
ELUTION PATTERN AND MS SPECTRA OF "PENNY" PEPTIDE BEFORE AND AFTER DEAMIDATION



ELUTION PATTERN AND MS^E SPECTRA OF T21 IN HEAVY-CHAIN BEFORE AND AFTER M-OXIDATION



MS^E SPECTRA OF DEAMIDATED "PENNY" PEPTIDES (AS SHOWN IN THE LEFT FIGURE)



CONCLUSIONS

1. **Peptide mapping with UPLC/MS^E is an approach that offers high resolution, high sensitivity, and unbiased identification. It's capable of separating, identifying and quantifying modifications in the antibody with stoichiometry above 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 the SYNAPT™ MS system ensures confident identification of modifications with small mass shifts (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 the SYNAPT™ MS system are an advanced platform for characterization of recombinant proteins, such as monoclonal antibodies.**

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