ADDING COST EFFECTIVE MASS DETECTION AS AN ORTHOGONAL TECHNIQUE FOR IMPROVED PRODUCTIVITY AND CONFIDENCE IN THE ANALYSIS OF PROTEIN BIOTHERAPEUTICS

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INTRODUCTION

Peptide analyses are frequently used during protein-based biotherapeutics development to assess critical quality attributes (CQAs) of the drugs. Throughout the development process, many optical based assays, developed following mass spectrometric characterization, are used to acquire product identity, assess purity, and monitor CQAs.

While optical detection provides a level of assurance, often times there is a need to add an orthogonal detection technique, such as mass detection, to improve specificity, expand detection limits, or increase confidence about peak homogeneity.

In this study, we present an analytical strategy for the acquisition of optical and MS based data for the efficient monitoring of CQAs associated with a mAb in a single cost-effective workflow.

Figure 1. *The ACQUITY® QDa. The compact footprint of the ACQUITY® QDa allows for convenient integration into laboratories for improved productivity. The straightforward user interface combined with disposable source elements minimizes training and maintenance for daily operation.*

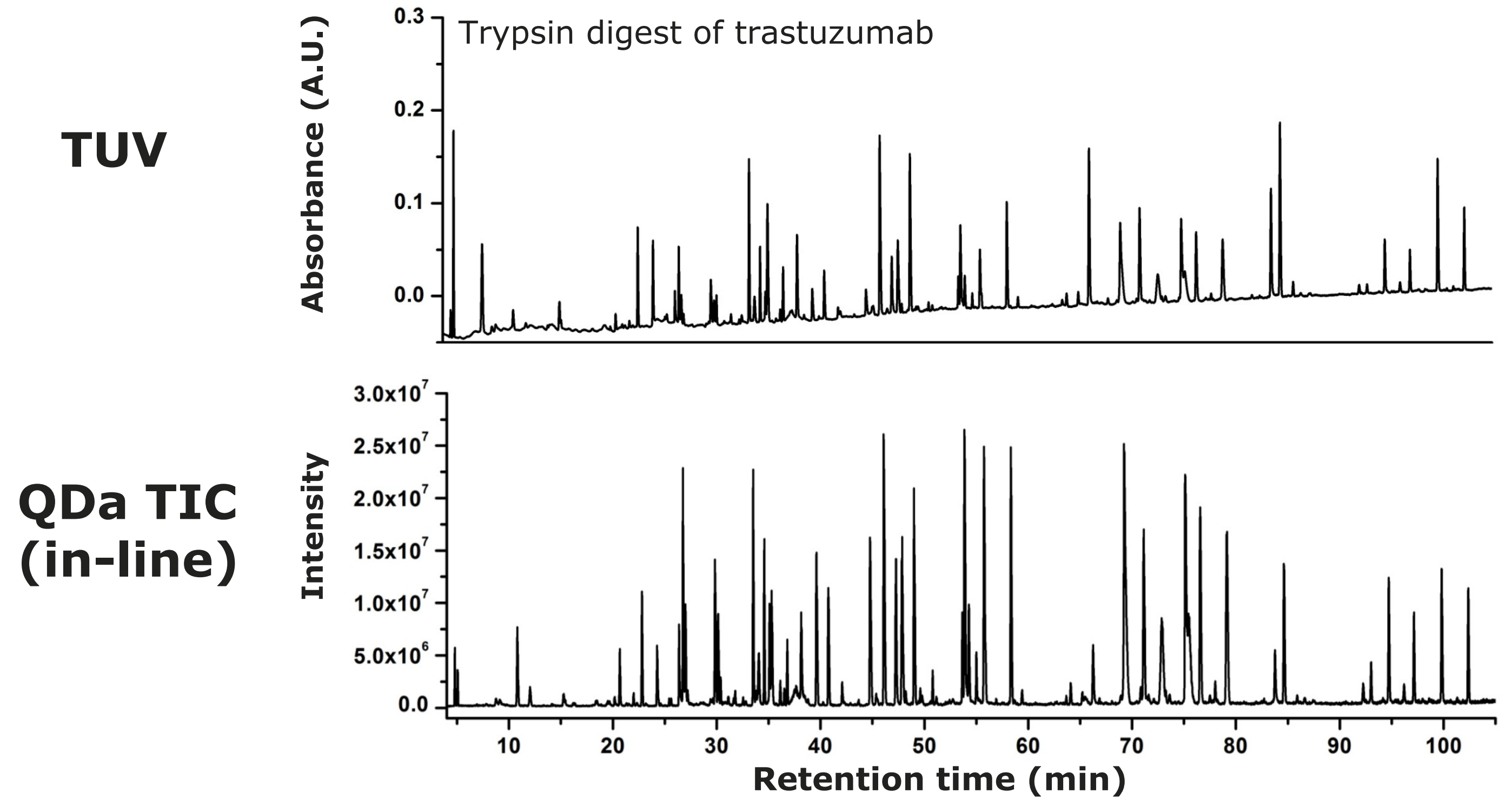


Figure 2. *In-line Orthogonal Detection. The ACQUITY® QDa combines straightforward mass spectral data with optical data for improving productivity and strengthening quality assurance in the biotherapeutic production environment.*

METHODS

LC Conditions:
LC System: ACQUITY UPLC® H-Class
Detectors: ACQUITY UPLC® TUV
Absorption Wavelength: 215 nm
CSH 130 Å C181.7 µm column, 2.1x100 mm
BEH300 Å C18 1.7 µm column, 2.1x100 mm
Column Temperature: 65 °C
Sample Temperature: 4 °C
Injection Volume: 8 µL (4 µg mass load)

ACQUITY® QDa Settings:
Sample rate: 2 points/sec
Mass range: 350 – 1250 Da.
Cone voltage: 10 V
Capillary voltage: 1.5 kV
Probe Temperature: 500 °C

Mobile phase:
A: H_2O, 0.1 % TFA
B: Acetonitrile, 0.1 % TFA
C: H_2O, 0.1 % FA
D: Acetonitrile, 0.1 % FA

Gradient table

Time	Flow (mL/min)	% A/C	% B/D
Initial	0.200	99	1
3.00	0.200	99	1
120.00	0.200	67	33
127.00	0.200	20	80
130.00	0.200	20	80
131.00	0.200	99	1
140.00	0.200	99	1

ACQUITY® QDa Evaluation: Fit-For-Purpose

The ACQUITY® QDa provides a simple and cost-effective solution for accurately detecting peptides across a wide molecular weight range and is fully compatible with traditional optical based LC peptide monitoring assays that incorporate trifluoroacetic acid (TFA) or formic acid (FA). Collectively, these results establish the ACQUITY® QDa is fit-for-purpose in routine peptide analysis assays.

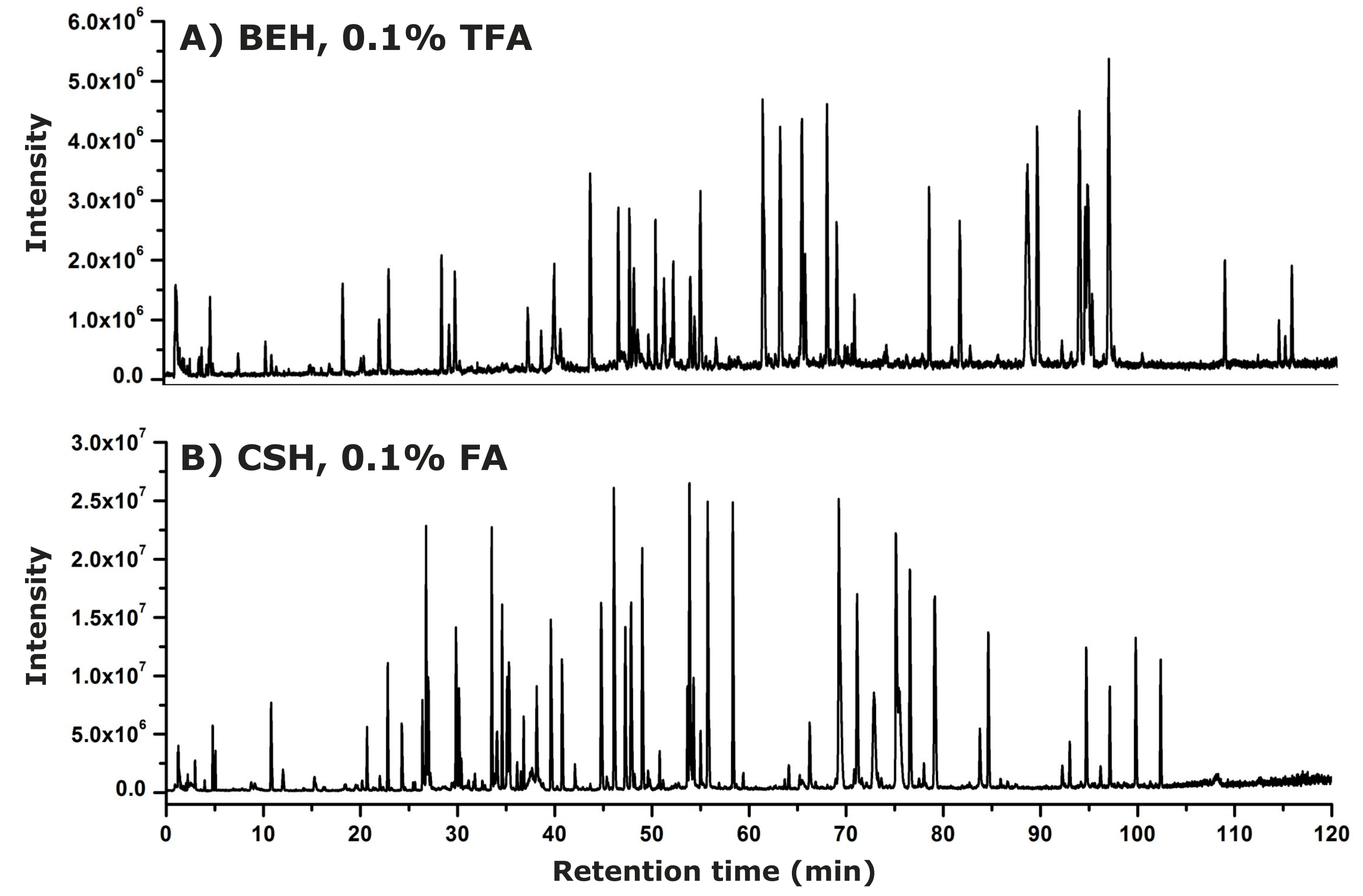
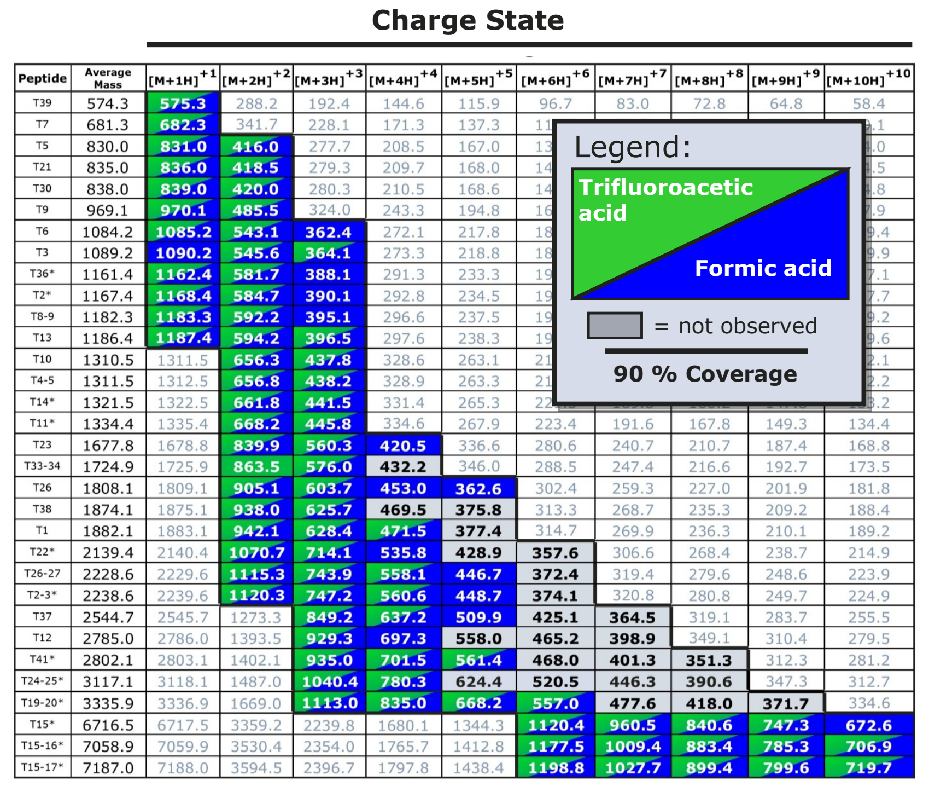


Figure 3. *Using the ACQUITY® QDa with Different Ion-pairing Agents. A 4µg sample of trypsin treated trastuzumab was separated with Waters RPLC column chemistry incorporating* ***A)*** *0.1% TFA, and* ***B)*** *0.1% FA in the mobile phases. Robust peptide profiles obtained under both conditions demonstrate the ACQUITY® QDa is capable of providing mass spectral data with conventional ion-pairing agents.*

Table 2. *Peptide Map Charge State Table. Multiple charge states observed for heavy chain tryptic peptides of trastuzumab using TFA and FA based methods, affords significant flexibility in method development of monitoring assays using the ACQUITY® QDa.*

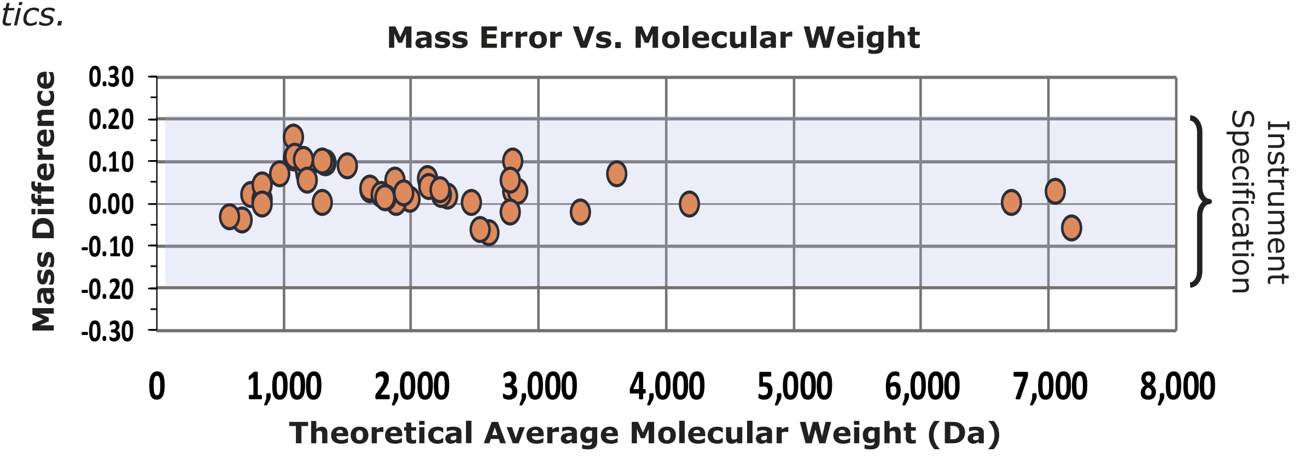


Charge State

Peptide	Average Mass	$[M+1H]^{+1}$	$[M+2H]^{+2}$	$[M+3H]^{+3}$	$[M+4H]^{+4}$	$[M+5H]^{+5}$	$[M+6H]^{+6}$	$[M+7H]^{+7}$	$[M+8H]^{+8}$	$[M+9H]^{+9}$	$[M+10H]^{+10}$
T39	574.3	575.3	288.2	192.4	144.6	115.9	96.7	83.0	72.8	64.8	58.4
T7	681.3	682.3	341.7	228.1	171.3	137.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T5	830.0	831.0	416.0	277.7	208.5	167.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T21	835.0	836.0	418.5	279.3	209.7	168.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T30	838.0	839.0	420.0	280.3	210.5	168.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T9	969.1	970.1	485.5	324.0	243.3	194.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T6	1084.2	1085.2	543.1	362.4	272.1	217.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T3	1089.2	1090.2	545.6	364.1	273.3	218.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T36*	1161.4	1162.4	581.7	388.1	291.3	233.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T2*	1167.4	1168.4	584.7	390.1	292.8	234.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T8-9	1182.3	1183.3	592.2	395.1	296.6	237.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T13	1186.4	1187.4	594.2	396.5	297.6	238.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T10	1310.5	1311.5	656.3	437.8	328.6	263.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T4-5	1311.5	1312.5	656.8	438.2	328.9	263.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T14*	1321.5	1322.5	661.8	441.5	331.4	265.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T11*	1334.4	1335.4	668.2	445.8	334.6	267.9	223.4	191.6	167.8	149.3	134.4
T23	1677.8	1678.8	839.9	560.3	420.5	336.6	280.6	240.7	210.7	187.4	168.8
T33-34	1724.9	1725.9	863.5	576.0	432.2	346.0	288.5	247.4	216.6	192.7	173.5
T26	1808.1	1809.1	905.1	603.7	453.0	362.6	302.4	259.3	227.0	201.9	181.8
T38	1874.1	1875.1	938.0	625.7	469.5	375.8	313.3	268.7	235.3	209.2	188.4
T1	1882.1	1883.1	942.1	628.4	471.5	377.4	314.7	269.9	236.3	210.1	189.2
T22*	2139.4	2140.4	1070.7	714.1	535.8	428.9	357.6	306.6	268.4	238.7	214.9
T26-27	2228.6	2229.6	1115.3	743.9	558.1	446.7	372.4	319.4	279.6	248.6	223.9
T2-3*	2238.6	2239.6	1120.3	747.2	560.6	448.7	374.1	320.8	280.8	249.7	224.9
T37	2544.7	2545.7	1273.3	849.2	637.2	509.9	425.1	364.5	319.1	283.7	255.5
T12	2785.0	2786.0	1393.5	929.3	697.3	558.0	465.2	398.9	349.1	310.4	279.5
T41*	2802.1	2803.1	1402.1	935.0	701.5	561.4	468.0	401.3	351.3	312.3	281.2
T24-25*	3117.1	3118.1	1487.0	1040.4	780.3	624.4	520.5	446.3	390.6	347.3	312.7
T19-20*	3335.9	3336.9	1669.0	1113.0	835.0	668.2	557.0	477.6	418.0	371.7	334.6
T15*	6716.5	6717.5	3359.2	2239.8	1680.1	1344.3	1120.4	960.5	840.6	747.3	672.6
T15-16*	7058.9	7059.9	3530.4	2354.0	1765.7	1412.8	1177.5	1009.4	883.4	785.3	706.9
T15-17*	7187.0	7188.0	3594.5	2396.7	1797.8	1438.4	1198.8	1027.7	899.4	799.6	719.7

Legend:
Trifluoroacetic acid
Formic acid
▨ = not observed
90 % Coverage

Figure 4. *Peptide Mass Accuracy. The ACQUITY® QDa is capable of providing mass information for peptides over a broad molecular weight range in assays routinely employed during the analysis of biotherapeutics.*



RESULTS AND DISCUSSION

Strengthening Confidence in the Biotherapeutic Environment

Improved productivity and lower development costs are often associated with the implementation of targeted analytical protocols to assess critical quality attributes (CQA) of biotherapeutics. The ACQUITY® QDa is a powerful tool that can increase productivity and confidence in data analysis within the biotherapeutic production environment when assessing product efficacy, identity, and purity.

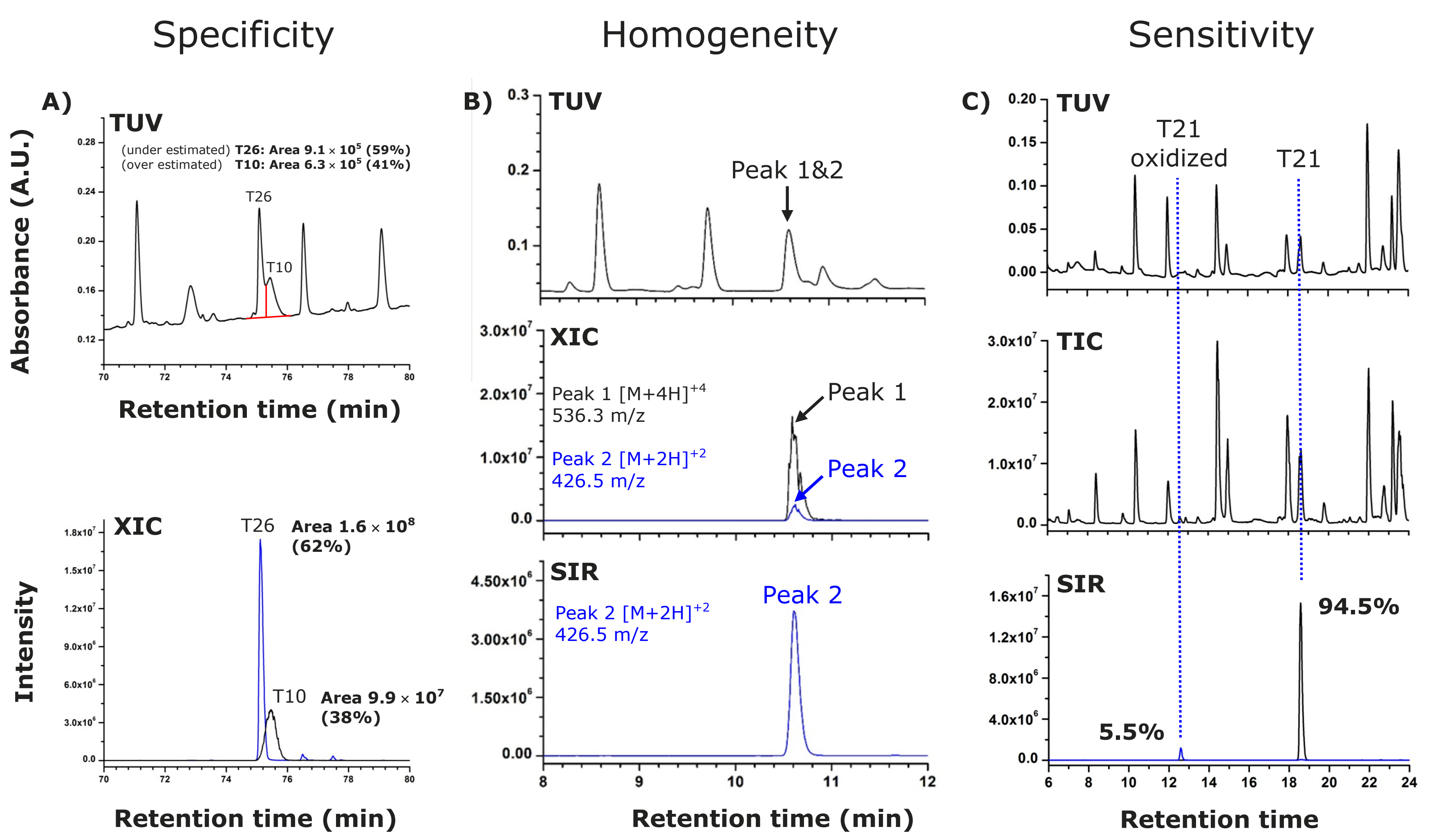
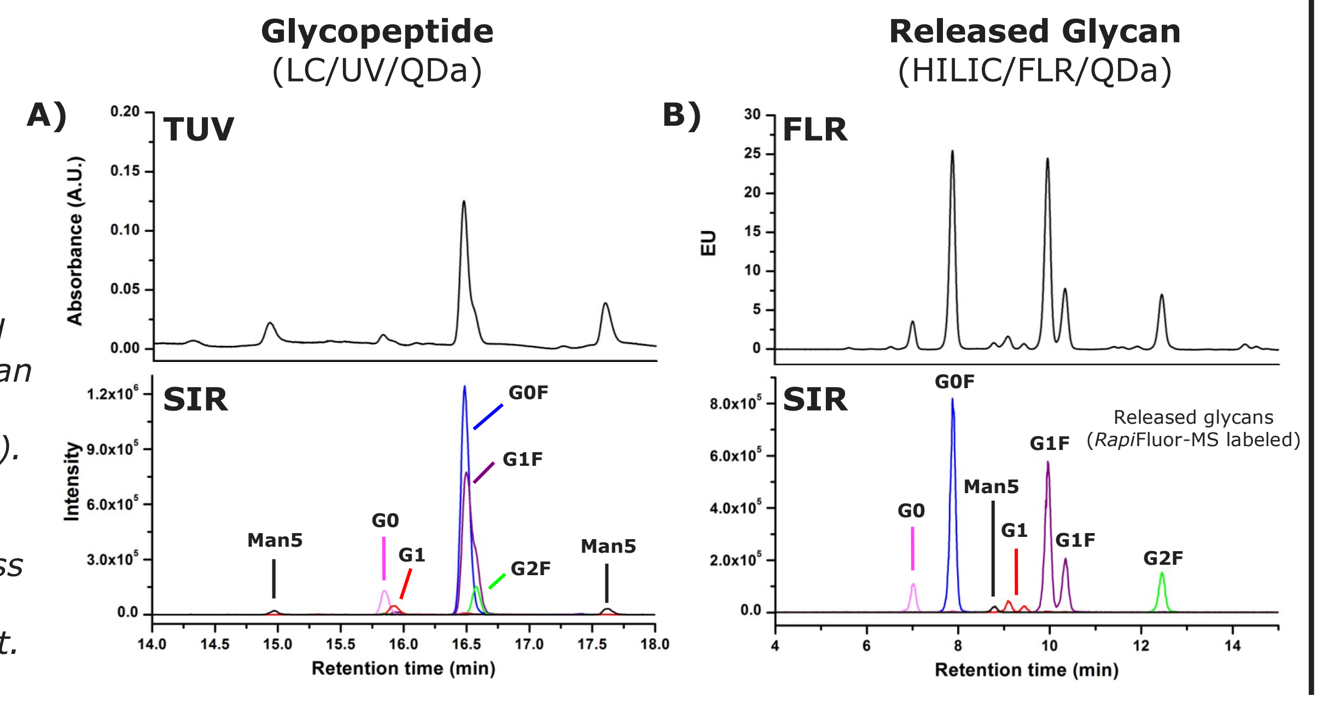
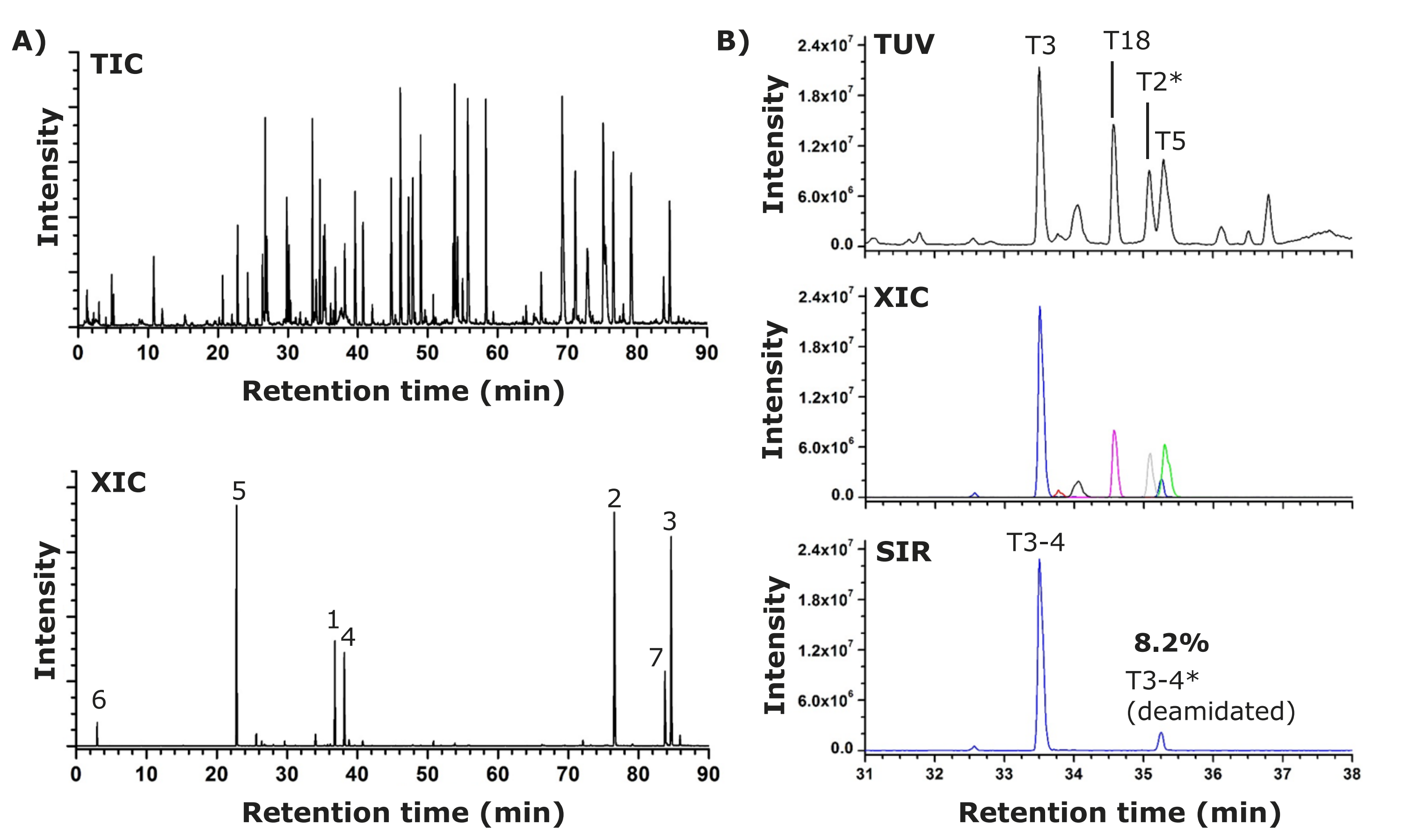


Figure 5. *Added Specificity and Sensitivity.* ***A)*** *The ACQUITY® QDa's ability to selectively monitor masses associated with each peptide enables increased accuracy in quantification of partially co-eluting species.* ***B)*** *The benefit of combining MS functionality with UV detection is evident in the assessment of peak homogeneity where UV detection alone can not distinguish between two perfectly co-eluting unique species. The added dimension of specificity the ACQUITY® QDa brings to routine monitoring assays is fully realized through the use of selected ion recording (SIR).* ***C)*** *Low abundant species, such as the oxidized form of the T21 peptide of trastuzumab, which affects binding efficiency, is not clearly defined with UV alone. Incorporation of SIRs minimizes baseline noise and ensures homogenous peaks are acquired for accurate quantification of low-abundant species that impact product efficacy and safety.*

Glycopeptide and Released Glycan Profiling

Figure 6. *Glycopeptide and released Glycan Profiling.* ***A)*** *Incorporation of SIRs using the ACQUITY® QDa enable analysts to monitor unique glycopeptide species that co-elute under RPLC conditions.* ***B)*** *Alternatively, n-linked glycans can be efficiently released and labeled (30 min) for separation using an ACQUITY UPLC® Glycan BEH Amide Column (130Å, 1.7 µm, 2.1 × 150 mm). Mass confirmation provided by the ACQUITY® QDa provides an efficient means for analysts to accurately assess and control factors that affect the biotherapeutic production environment.*





CDR Peptides

Light Chain
1.ASQDVNTAVAWYQQKPGK
2.LLIYSASFLYSGVPSR
3.SGTDFTLTISSLQPEDFATYY-
-CQQHYTTPPTFGQGTK

Heavy Chain
4. DTYIHWVR
5. IYPTNGYTR/(6.)YADSVKG
7. WGGDGFYAMDYWGQGTLV-
-TVSSASTK

CDR Deamidation

T3-4: ASQDVNTAVAWYQQKPGKAPK

	Peptide	Average Mass (Da)	XIC m/z
	T3-4	2287.6	572.9
	T18*	1,876.1	626.3
	T2*	1,167.3	584.6
	T5	829.9	415.9
*	T3-4*	2,288.5	573.1

* T3-4 deamidated form

Figure 7. *Drug Identity and Efficacy. The ACQUITY® QDa offers an efficient method that can be readily adapted to existing workflows for identity screening assays for improved productivity.* ***A)*** *Complementary determining region (CDR) peptide profiles unique to biotherapeutic drug products are efficiently extracted from the full MS scan of a peptide map of trastuzumab using the ACQUITY® QDa for the rapid determination of product identity.*
B) *Furthermore, deamidation events in CDR containing peptides (T3-4*), which can impact binding efficiency, are readily monitored for quantification using SIRs despite their co-elution with unrelated peptides, demonstrating the ACQUITY QDa's ability to provide an added level of quality assurance in the biotherapeutic production environment.*

CONCLUSION

- Detect and monitor peptides over wide MW range
- Quantify peptide variants with enhanced specificity
- Selectively detect and monitor co-eluting components
- Increase productivity and confidence in data analysis within existing workflows