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Note d'application

# Monitoring Multiple Attributes in a Single Assay Using the ACQUITY QDa Detector for Product Confirmation and Process Monitoring of Product Quality Attributes

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## Abstract

This application note reveals a proof of concept strategy for identifying multiple product attributes within a single study using the ACQUITY QDa Detector with compliant-ready chromatographic data software such as Empower.

The ACQUITY QDa Detector provides an efficient and cost-effective solution for monitoring important product attributes in a development or QC environment when characterization has been previously carried out using a high resolution MS instrument. This application note demonstrates that CDR peptides, oxidized and deamidated peptides, and glycopeptides can be identified, quantitated, and reported from a single acquisition using the derived channel and inter-channel calculation functionalities in the Empower software. The list of attributes to be monitored would need to be determined for each respective antibody, but could be readily expanded to meet the individual user's needs.

## Benefits

- Multiple attribute monitoring through a single acquisition to enable both product confirmation and routine

screening of post-translational modifications

- Empower Software enables an automated and compliant workflow for data acquisition, processing, and reporting of multiple product quality attributes

## Introduction

The concept of using a single LC-MS-based analytical method to monitor multiple product quality attributes (PQAs) is a strategy that has started to gain momentum in the biopharmaceutical industry. The idea behind this concept is that a single LC-MS method can be used to assess a product's important quality attributes simultaneously, as opposed to running a panel of optically-based chromatographic methods, which are unable to assess product attributes at a molecular level. The motivation for extending mass spectrometry (MS) beyond characterization into all stages of development and even quality control, is largely due to the complexity of protein-based therapeutics compared to small molecule drugs.[1] Replacing conventional chromatographic methods with a single, more sophisticated LC-MS method ultimately provides greater product and process understanding, which is required to support Quality by Design (QbD) regulatory submissions. Implementation of QbD is encouraged by regulatory agencies as a way of improving product quality and patient safety by offering a systematic and proactive approach to product development.[2,3]

It has recently been shown that a method for identity testing which monitors the complementarity determining region (CDR) peptides of a monoclonal antibody (mAb) has been developed and validated using the ACQUITY QDa Detector.[4] Using this work as the foundation of our study, we consider the need for a single test to be used for both product confirmation and also for monitoring a number of pre-characterized post-translational modifications (PTMs) using the ACQUITY QDa Detector, which provides a cost-effective solution for incorporating mass data into analysis. To align with the published work, a sample of trastuzumab and Waters Intact mAb Standard can be checked against the CDR peptides of a trastuzumab reference standard to verify method specificity through retention time and mass determination. Because a drug product's critical quality attributes (CQAs) would need to be independently determined, the current study is meant to serve as a proof of concept for providing a strategy for identifying multiple attributes within a single study using the ACQUITY QDa Detector with compliant-ready chromatographic data software such as Empower.

# Experimental

## LC Conditions

| | |
|---|---|
| LC system: | ACQUITY UPLC H-Class Bio |
| Detectors: | ACQUITY UPLC TUV<br>ACQUITY QDa Detector (Performance Model) |
| Absorption wavelength: | 215 nm |
| Column: | ACQUITY UPLC Peptide CSH $C_{18}$ 130 Å, 1.7 µm, 2.1 mm x 100 mm |
| Column temp.: | 65 °C |
| Mobile phase A: | H2O with 0.1% (v/v)<br>formic acid |
| Mobile phase B: | Acetonitrile with 0.1% (v/v) formic acid |
| Sample temp.: | 10 °C |
| Injection volume: | 10 µL |

## Gradient

| Time (min) | Flow rate (mL/min) | %A | %B | %C | %D |
|---|---|---|---|---|---|
| Initial | 0.2 | 97 | 3 | 0 | 0 |

| Time (min) | Flow rate (mL/min) | %A | %B | %C | %D |
|---|---|---|---|---|---|
| 3 | 0.2 | 97 | 3 | 0 | 0 |
| 120 | 0.2 | 67 | 33 | 0 | 0 |
| 127 | 0.2 | 20 | 80 | 0 | 0 |
| 130 | 0.2 | 20 | 80 | 0 | 0 |
| 131 | 0.2 | 97 | 3 | 0 | 0 |
| 150 | 0.2 | 97 | 3 | 0 | 0 |

## Detector Settings

| | |
|---|---|
| Sampling rate: | 2 Hz |
| Mass range: | 350–1250 Da |
| Ionization mode: | ESI+, centroid |
| Cone voltage: | 10 V |
| Capillary voltage: | 1.5 kV |
| Probe temp.: | 500 °C |

## Data management

Empower 3 CDS, SR2

## Results and Discussion

### Monitoring Multiple Attributes using Extracted Ion Chromatograms for Identity Confirmation

The variable region of an antibody contains CDR peptides that are unique to that specific antibody, which allows these peptides to be used for identification purposes. To begin the evaluation of the ACQUITY QDa Detector for reporting multiple attributes, we began by collecting a  peptide map of trastuzumab. A trypsin digest of reduced and alkylated trastuzumab was prepared and injected at a final concentration of approximately 0.5 mg/mL without further dilution. The peptide mapping method described above was used with the ACQUITY QDa Detector set to collect a full scan so that extracted ion chromatograms (XICs) could be used to identify the attributes of interest. Figure 1 shows a strong correlation between the optical trace and the corresponding mass data. This data suggests that the ACQUITY QDa Detector provides an effective way to incorporate mass measurements into an LC-UV based peptide map assay.

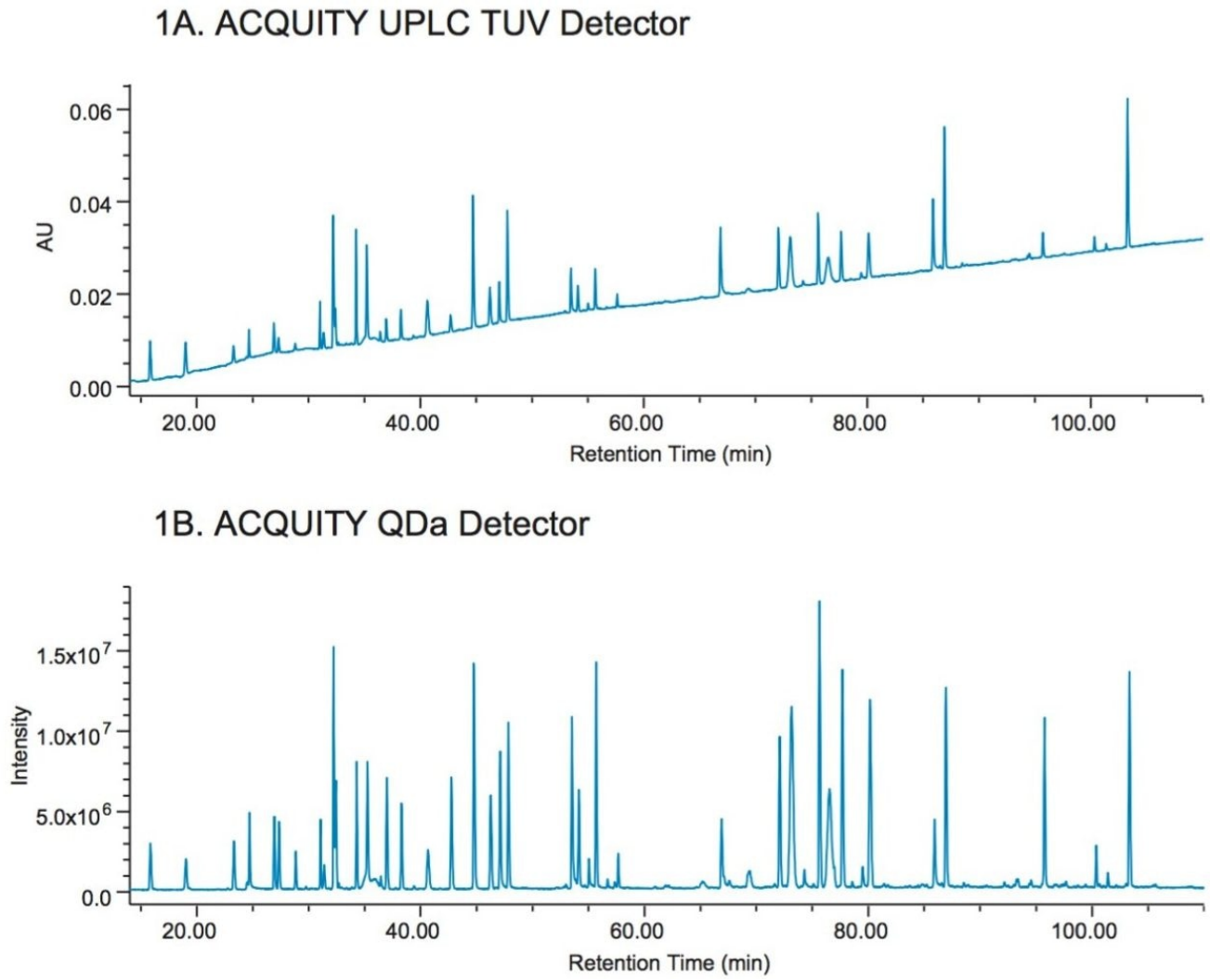



*Figure 1. Peptide map detection. 1A) Optical detection of a trypsin digest of trastuzumab. 1B) Corresponding ACQUITY QDa data.*

The CDR peptides can be used to confirm the identity of a given antibody by using XICs to extract the *m/z* of each CDR peptide. To avoid manual integration of each of the respective XIC channels, derived channels can be used. The process for creating derived channels has been previously described.[5] In brief, a method set can be created to contain a derived channel containing the *m/z* of each of the CDR peptides. This will essentially extract the *m/z* of each of the desired peptides into a single channel. By linking the derived channel to a processing method with associated retention time and component labels, the result is a single channel containing each of the CDR peptides which are now identified according to mass and retention time. Figure 2A shows a screen

capture from Empower that illustrates how a method set can be created to contain derived channels, where a new derived channel can be created for each of the attributes of interest. In the case of the CDR peptides, the mass of six CDR peptides can be entered into the Formula field of the derived channel as shown in Figure 2B. In this example, a single dominant charge state is used to identify each of the CDR peptides, but the user could sum over additional charge states if desired.

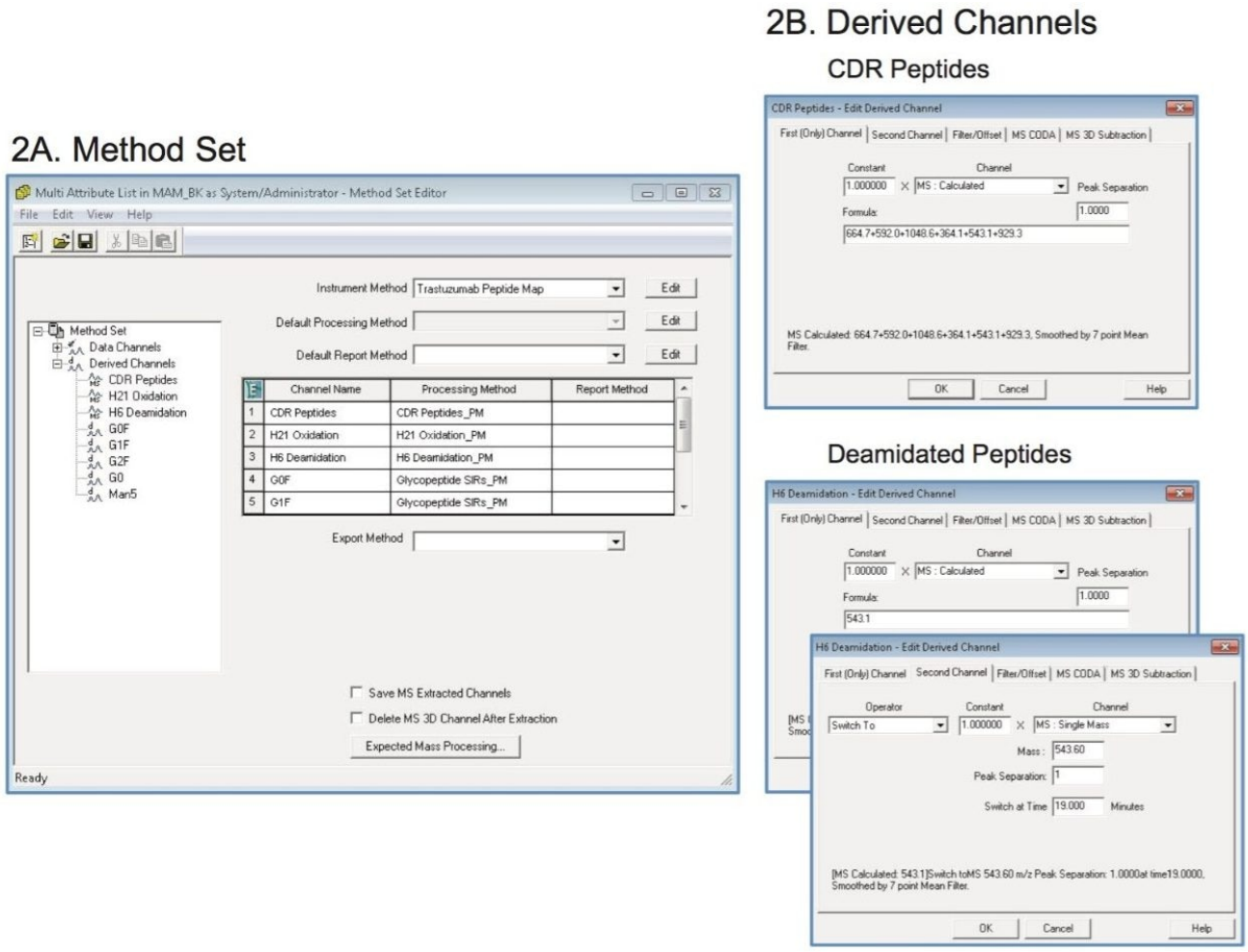



*Figure 2. Empower screen captures. 2A) A method set containing derived channels used to determine if previously characterized attributes are present in a sample. The attributes of interest in this case are CDR peptides, an oxidized peptide, a deamidated peptide, and glycopeptides. Each attribute has an independent processing method associated with it. Attributes to be quantitated using XICs can be monitored through a single derived channel for that attribute, while attributes to be quantitated using SIRs must be monitored through individual channels and quantitated through custom calculations. 2B) Examples of derived channels. To create a derived channel for CDR peptides, the m/z of each peptide can be entered into the formula field. Six CDR peptides are monitored in this example. For the deamidated peptide, a time switch can be entered to switch from one m/z to another at a given time. This avoids signal overlap from using XICs having only a small difference in mass between the native and modified peptides.*

The average mass for each of the CDR peptides is reported in Table 1 as well as the calculated m/z values used for this study. This table also contains mass information for additional attributes to be monitored as discussed below. The chromatogram resulting from the derived channel can be seen in Figure 3A. In this figure, XICs of each of the six CDR peptides are clearly identified, which confirms sample identity.

## Monitoring Multiple Attributes using Extracted Ion Chromatograms for Process Monitoring

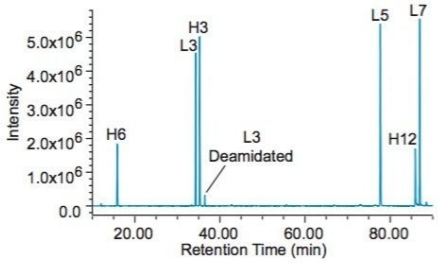
A similar approach can be used to quantify chemical modifications, such as deamidation or oxidation. Process changes during manufacturing or storage conditions can affect the rate of modification, which could potentially impact antibody activity or antigen binding.[6] In this example, we consider asparagine deamidation and methionine oxidation. Again, we make the assumption that these modifications would have been characterized using high resolution MS and determined to be important quality attributes.

Because the mass difference of the oxidation modification is large, this modification can be tracked in derived channels in a similar manner as the CDR peptides. The resulting XICs can be seen in Figure 3B. The deamidation event, however, shows a much smaller difference in mass between the native and deamidated peptides, so the data must be treated in a different way to reliably determine the relative abundance of each of the peptides. In this case, because the peaks are chromatographically resolved, a derived channel can be used that switches from one calculated mass to another at a time established by the user. This process is more clearly illustrated in Figure 2B. From the Empower screen captures, the mass to be monitored switches from $m/z$=543.1 Da (native peptide, z=2) to 543.6 Da (deamidated peptide, z=2) at 19 minutes. Using a second channel avoids signal overlap from the native and deamidated species. The XICs of the native and deamidated peptide can be seen in Figure 3C.

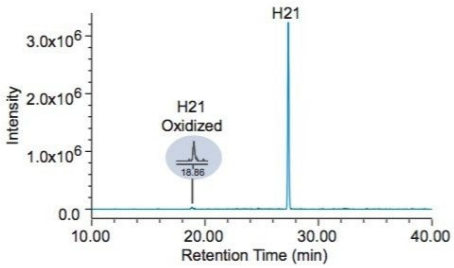
| Peptide | Identification/ Modification | Average mass (Da) | Charge state | Calculated (*m/z*) |
|---|---|---|---|---|
| L3 | CDR | 1991.17 | $[M+3H]^{+3}$ | 664.7 |
| L5 | CDR | 1773.04 | $[M+3H]^{+3}$ | 592.0 |
| L7 | CDR | 4190.48 | $[M+4H]^{+4}$ | 1048.6 |
| H3 | CDR | 1089.21 | $[M+3H]^{+3}$ | 364.1 |
| H6 | CDR | 1084.18 | $[M+2H]^{+2}$ | 543.1 |
| H6 | Deamidation | 1085.17 | $[M+2H]^{+2}$ | 543.6 |
| H12 | CDR | 2785.01 | $[M+3H]^{+3}$ | 929.3 |
| H21 | Native | 834.43 | $[M+2H]^{+2}$ | 418.2 |
| H21 | Oxidation | 850.42 | $[M+2H]^{+2}$ | 426.2 |
| H25 | G0F | 2634.53 | $[M+3H]^{+3}$ | 879.2 |
| H25 | G1F | 2796.67 | $[M+3H]^{+3}$ | 933.2 |
| H25 | G2F | 2958.81 | $[M+3H]^{+3}$ | 987.3 |
| H25 | G0 | 2488.39 | $[M+3H]^{+3}$ | 830.5 |
| H25 | G1 | 2650.53 | $[M+3H]^{+3}$ | 884.5 |
| H25 | Man5 | 2406.28 | $[M+3H]^{+3}$ | 803.1 |

*Table 1. Peptide information for reported attributes.*

3A. CDR Peptides, XICs




3B. Oxidized Peptide, XICs




3C. Deamidated Peptide, XICs

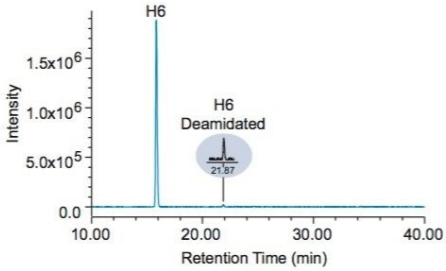



*Figure 3. XICs used to identify attributes of interest. Peak labels "H" and "L" refer to heavy chain and light chain peptides, respectively. 3A) The CDR peptides unique to trastuzumab. 3B) A native peptide and its oxidized form. 3C) A native peptide and its deamidated form. Note that the native peptide, H6, is also a CDR peptide. This peptide can function to establish identity but can also be used along with its deamidated form to quantitate the percent modification independent of the CDR channel. Insets in 3B. and 3C. show a 10X zoomed in image of the oxidized and deamidated peptides, respectively. Signal-to-noise is well above minimum requirements to reliably quantitate these low level modifications.*

## Monitoring Multiple Attributes using Selected Ion Recording for Process Monitoring

When additional specificity and sensitivity are needed for peak monitoring, selected ion recording (SIR) can be used. By incorporating SIRs, a single *m/z* is selected and passed through to the detector. To demonstrate the utility of SIRs, the dominant charge state of the five most abundant glycopeptides (G0F, G1F, G2F, G0, and Man5) was previously determined. Figure 4 shows an overlay of the five SIR channels.

For monitoring glycopeptides, the additional sensitivity afforded by the SIRs is required for accurate quantitation. Because each SIR is associated with its own channel, custom calculations can be used to calculate the relative abundance of each glycopeptide.

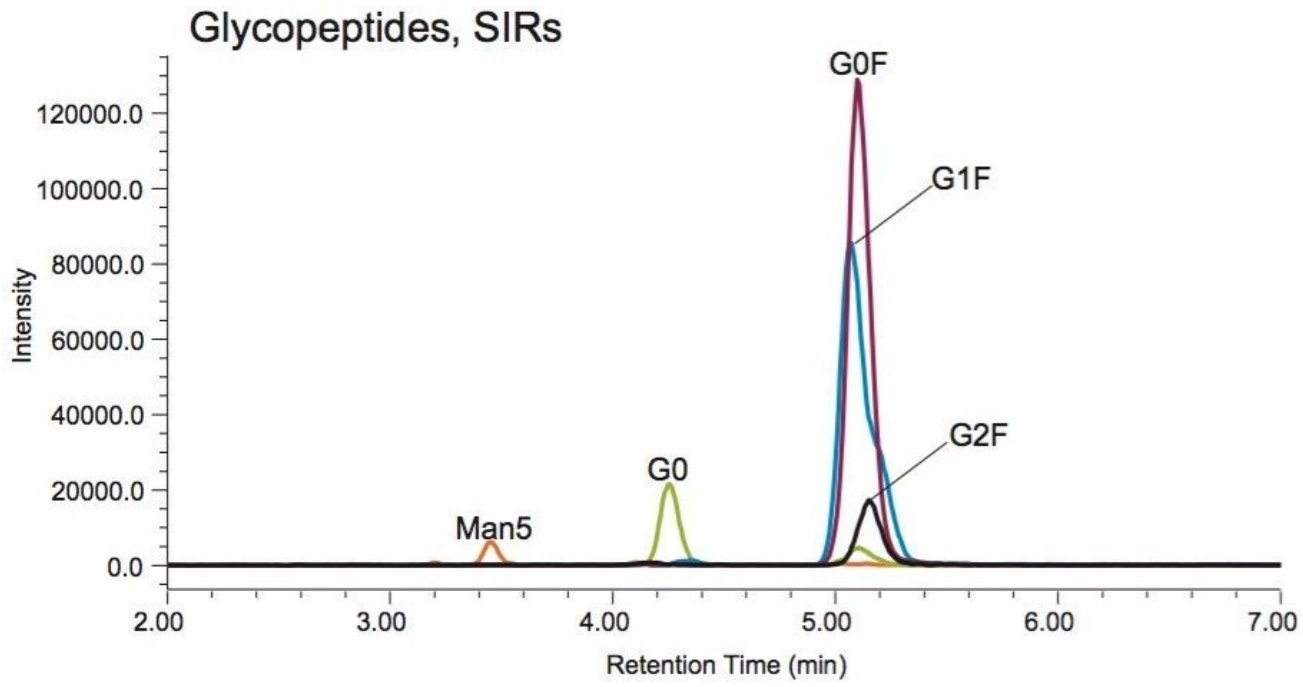



*Figure 4. Overlay of five SIR channels used for determining relative abundance of five glycopeptides. Because each SIR is collected in an individual channel, custom calculations must be used to automate the process of determining relative abundance of each glycopeptide.*

## Empower Software Enables Automated Reporting of Multiple Attributes

We have just shown how a method set can be created to contain derived channels used to monitor attributes of interest, each of which can have an independent processing method associated with it. This same method set can be used to associate independent reporting methods for each of the attributes of interest, which aids to further automate the monitoring process. Should a user wish to import all results into a single report, Empower

can accommodate this functionality as well. Figure 5 shows screen captures of Empower reports generated to monitor each of the attributes previously discussed.

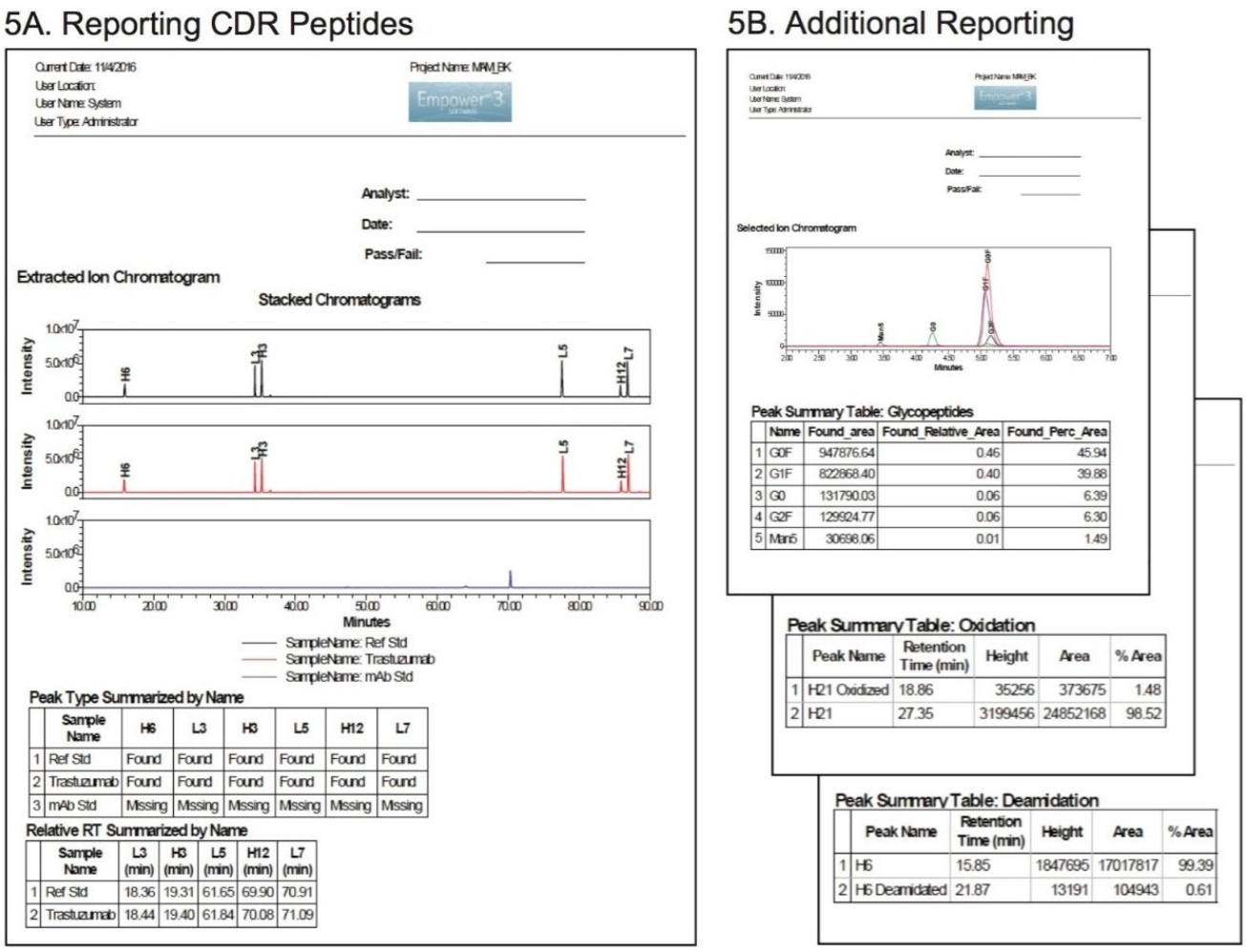



*Figure 5. Empower reporting. 5A) Comparing a reference standard, a sample of trastuzumab, and an intact mAb standard (negative control). Peak tables summarize whether the peaks of interest were detected and the relative retention time for each CDR peptide. In lieu of an internal standard, H6 was used to calculate relative retention time. 5B) Additional reporting for a sample of trastuzumab. Each of the items reported is the result of designing a processing method and derived channel for that specific attribute. In reporting relative abundance of the glycopeptides, Found_area, Found_Relative_Area, and Found_Perc_Area are defined by the custom calculations functionality in Empower. These fields are created so that peak area from individual channels can be reported relative to one another.*

## Conclusion

The ACQUITY QDa Detector provides an efficient and cost-effective solution for monitoring important product attributes in a development or QC environment when characterization has been previously carried out using a high resolution MS instrument. This application note demonstrates that CDR peptides, oxidized and deamidated peptides, and glycopeptides can be identified, quantitated, and reported from a single acquisition using the derived channel and inter-channel calculation functionalities in the Empower software. The list of attributes to be monitored would need to be determined for each respective antibody, but could be readily expanded to meet the individual user's needs.

## References


1. Arnaud, C. H. Mass Spec Weighs in on Protein Therapeutics. *C&EN*. 2016; 94(22): 30–34.
2. FDA, Analytical Procedures and Methods Validation for Drugs and Biologics. 2015.
3. ICH, Q8-Q12. (accessed November 2016).
4. Zhang, J. et al. Development and Validation of a Peptide Mapping Method for the Characterization of Adalimumab with QDa Detector. *Chromatographia*. 2016; 79(7): 395–403.
5. Birdsall, R. E., McCarthy, S. M. Increasing Specificity and Sensitivity in Routine Peptide Analyses Using Mass Detection with the ACQUITY QDa Detector. 2015; 720005377.
6. Haberger, M. et al. Assessment of Chemical Modifications of Sites in the CDRs of Recombinant Antibodies: Susceptibility vs. Functionality of Critical Quality Attributes. *mAbs*. 2014; 6(2):327–339.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ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>

Empower Chromatography Data Software <https://www.waters.com/10190669>

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