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Applikationsbericht

Extending the Analytics of Biopharmaceutical QA/QC Labs with the ACQUITY™ QDa™ II Mass Detector

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Abstract

The ACQUITY QDa II Mass Detector, the next generation of compact mass detectors, is specifically engineered for sustainable lab practices and features several enhancements over its predecessor, including an extended mass range, simplified maintenance, as well as improved robustness & consistency in data. In this study, the ACQUITY QDa II Mass Detector offers superior sensitivity and specificity over UV methods. It is well-equipped of supporting various biopharmaceutical manufacturing activities, such as raw material testing, impurity monitoring, and identity testing as part of cGMP. With its compact design, on/off simplicity, and integrated Empower™ 3 Software control, the ACQUITY QDa II Mass Detector is well positioned to reduce operating costs and improve productivity of support labs in the manufacturing of biopharmaceuticals.

Benefits

- Empower 3 Software control offers compliant MS solution for regulated labs
- Extended mass range from 30–1500 *m/z* increases assay sensitivity for critical species
- Ability to detect impurities as low as 0.01% (v/v) as part of regulatory guidance

- Consistent and reliable results for increased accuracy and assay robustness

Introduction

Research into biopharmaceuticals as therapeutics to treat cancer and rare diseases continues to exhibit steady growth within the pharmaceutical industry. Protein-based drug therapies, such as monoclonal antibodies (mAbs), represent some of the most successful biological therapies to date. Their success can be partly attributed to their ability to be tailored toward diseases while minimizing adverse side effects making them superior in specificity and safety over traditional treatment methods.[1] Proteins, which are comprised of amino acids, derive their function from the order and composition of these amino acids. Changes in the amino acids or their sequence can impact a protein's functionality. As with all pharmaceuticals, regulatory bodies expect a comprehensive control strategy in manufacturing environments. This strategy is designed to monitor known product/process related impurities and critical quality attributes associated with the drug product before its release to ensure drug products are safe and efficacious.

Due to their complex structure, mAbs can be enzymatically treated to cleave the primary amino acid sequence at specific locations to produce smaller peptide fragments. The intrinsic properties of peptides enable analysis with techniques such as reversed-phase liquid chromatography (RPLC). The need for enhanced analytics in research and development phases often results in regulated labs receiving MS-based methods from upstream characterization and/or process related activity. This can present challenges in QC environments as UV-based method may not provide equivalent sensitivity or specificity for monitoring impurities, thus potentially requiring additional resources for further method development as part of qualification/validation activity. The ACQUITY QDa II Mass Detector (Figure 1) represents the next generation of compact mass detectors integrated with Waters Empower 3 Software to offer a QC-friendly solution to help reduce the burden associated with costly method development while facilitating easier transfer and deployment of MS-based methods in regulated environments.

In this study, we evaluate the performance and utility of the ACQUITY QDa II Mass Detector in the deployment of common assays performed as part of manufacturing activity of biopharmaceuticals. The metrics to be investigated include assay sensitivity, specificity, and reproducibility.

Figure 1. The ACQUITY QDa II Mass Detector represents the next generation of compact mass detectors engineered for sustainable lab practices featuring an extended mass range, improved robustness, and consistency increasing analytical capabilities of supporting labs while maintaining its on/off simplicity for flexible deployment.

Experimental

Sample

Waters mAb Tryptic Digestion Standard (p/n: 186009126) < https://www.waters.com/nextgen/global/shop/standards--reagents/186009126-mab-tryptic-digestion-standard.html> was reconstituted in LC-MS grade water with 0.1% formic acid to yield a final concentration of 0.4 mg/mL, 3 µL (1.2 µg) sample was loaded for each injection.

LC System Conditions

LC system:	ACQUITY Premier System (BSM variant)
Detection:	Ti FC, λ= 220 nm
Column:	ACQUITY Premier Peptide CSH™ C_{18} Column, 130 Å, 1.7 µm, 2.1 x 100 mm (p/n: 186009488)
Column temperature:	60 °C
Sample temperature:	10 °C
Injection volume:	3 µL
Flow rate:	0.200 mL/min
Mobile phase:	A1: Water, 0.1% FA B1: Acetonitrile, 0.1% FA
Chromatography software:	Empower 3.8.1

Gradient Table

Time (min)	Flow rate (mL/min)	%A	%B	Curve
initial	0.200	99.0	1.0	initial
3.00	0.200	99.0	1.0	6
42.00	0.200	60.0	40.0	6
45.00	0.200	35.0	65.0	6
48.00	0.200	35.0	65.0	6
51.00	0.200	99.0	1.0	6
70.00	0.200	99.0	1.0	6

MS Detector Settings

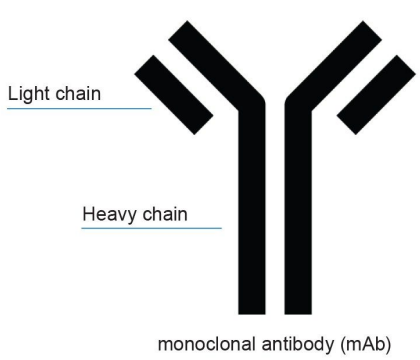
MS detector:	ACQUITY QDa II Mass Detector
Scan mode:	Positive Electrospray (+)
Scan rate:	5 Hz
Scan range:	350–1500 *m/z*
Probe temperature:	600 °C
Capillary voltage:	1.5 kV
Cone voltage:	20 V

SIRs

CDR Peptide fragment	*m/z*
LC T2	541.6, 1082.2
LC T3	406.5, 541.6
LC T5	477.1, 953.1
LC T6	393.9, 786.9
LC T7	1122.2, 1495.9
HC T2	926.6, 1235.1
HC T4	554.6, 831.4
HC T12	935.0, 1402.1
HC T6	430.0, 858.9
CQA Peptide fragment	***m/z***
T21	418.5, 836.0
T21 Ox	426.5, 852.0
T37 native	849.2, 1273.3
T37 deamidated	849.6, 1273.8
T37 succinimide	843.6, 1264.8

Results and Discussion

One application that benefits from the specificity afforded by the ACQUITY QDa II Mass Detector is the identity testing of raw materials or drug substances/products as part of current Good Manufacturing Practices (cGMP). As part of lab transfers and compliance, assays that establish sample identity are essential to ensure drug product consistency during routine analyses.[2] As shown in Figure 2, mAbs contain specific amino acid residues that are unique to its primary structure and function. These complementary determining region (CDR) sequences, which enable the mAb to bind to epitopes of target cells, act as a "fingerprint" and are frequently used in peptide-based identity tests. When enzymatically treated, the CDR sequences comprise a set of unique peptides as shown in the table of Figure 3A. As part of the instrument control within the Empower 3 Software, the ACQUITY QDa II Mass Detector has the ability to use selected ion recording (SIR) functionality to monitor ions associated with the CDR containing peptides as part of identity testing (Figure 3A, bottom panel).



Light Chain (LC) sequence

DIQMTQSPST LSASVGDRVT ITC**SASSRVG** **YMH**WYQQKPG KAPKLLIY**DT** **SKLAS**GVPSR
FSGSGSGTEF TLTISSLQPD DFATYYC**FQG** **SGYPFT**FGGG TKVEIKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Heavy Chain (HC) sequence

PQVTLRESGP ALVKPTQTLT LTCTFSGFSL S**TAGMSVG**WI RQPPGKALEW LA**DIWWDDKK**
HYNPSLKDRL TISKDTSKNQ VVLKVTNMDP ADTATYYCAR **DMIFNFYFDV** WGQGTTVTVS
SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS
SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCPAPELLG
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE
EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR
WQQGNVFSCS VMHEALHNHY TQKSLSLSPG

Figure 2. Primary sequences for the light chain and heavy chain of the Waters mAb Tryptic Digestion Standard (p/n: 186009126). Amino acid residues associated with the complementary determining region are highlighted red.

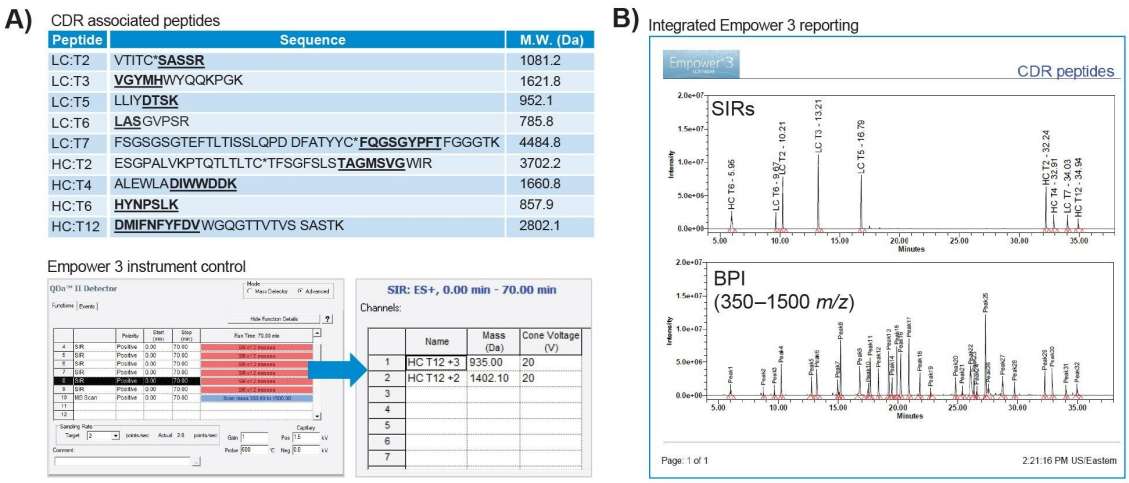


Figure 3. A) Peptide fragments associated with CDR sequences can be monitored with the ACQUITY QDa II Mass Detector using the integrated SIR functionality within the Empower 3 instrument method settings. B) Chromatograms of CDR containing peptides can be readily annotated and reported as part of cGMP.

As shown in Figure 3B, the ACQUITY QDa II Mass Detector was more than capable of detecting all the CDR containing peptides using the integrated SIR functionality within the Empower 3 Software instrument method. In this example, SIRs related to each CDR containing peptide were acquired along with a full scan of the Waters mAb Tryptic Digestion Standard. Notably, one of the new features of the ACQUITY QDa II Mass Detector is the extended mass range, which now supports the detection of ions with a mass-to-charge ratio up to 1500 *m/z*, compared to the previous generation (Figure 4A). As shown in Figure 4B, this resulted in up to a 50% increase in MS response of the larger CDR containing peptides which exhibited ions in the extended mass range. This instrument enhancement can be particularly useful for improved assay sensitivity toward larger peptides, which result from missed cleavages or the use of alternative enzymes such as ASP-N.

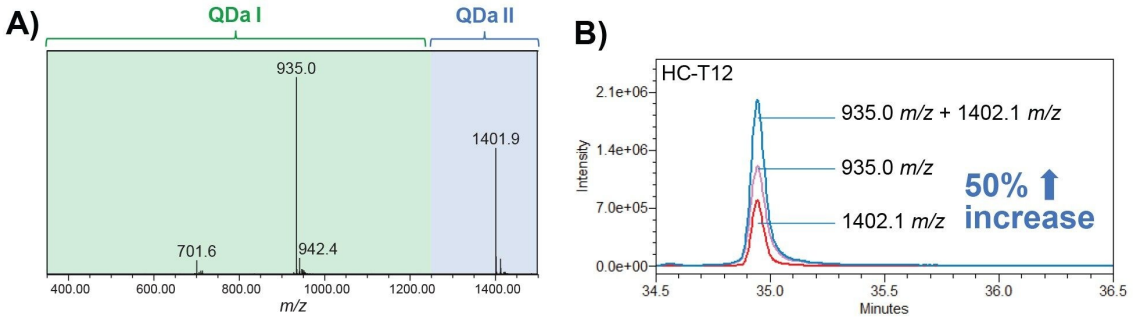


Figure 4. A) The extended mass range of the ACQUITY QDa II Mass Detector enables detection of ions associated with peptide fragments above 1250 m/z when compared to original QDa. B) MS response was observed to increase up to 50% for CDR peptides that exhibited ions above 1250 m/z.

The ability to extract ion chromatograms (XICs) post-acquisition or target specific masses associated with peptides during acquisition for increased sensitivity (SIRs) is well suited for general impurity screening, critical quality attributes (CQA) monitoring, or supporting multi-attribute monitoring (MAM) methods. An example of the former is shown in Figure 5A. In this example, the T21 peptide fragment (sequence: DTLMISR), which is well known as a peptide sensitive to oxidation, was monitored using SIRs along with its oxidized form (T21 Ox). In this instance, the oxidized impurity was readily determined to account for 4% of the peak area relative to its native form in the commercial digest sample. While this demonstrates its ability to monitor peptide impurities, further investigation of the limit of detection (L.O.D.) of the ACQUITY QDa II Mass Detector was performed using a synthetic standard. Briefly, a synthesized peptide representing the T21 oxidized impurity was normalized against the native species in the commercial digest using peak area acquired under UV detection. Once normalized, the synthetic impurity was diluted up to 10,000-fold to represent an impurity present at 0.01%. As shown in Figure 5B, the ACQUITY QDa II Mass Detector was able to detect the impurity well below UV limits with a detection limit of 0.01%, exhibiting a signal-to-noise ratio (S/N) of approximately three (top panel). These results indicate that the detection capabilities of the ACQUITY QDa II Mass Detector are more than sufficient to monitor impurities at regulatory reporting thresholds (0.05%). This further demonstrates the utility that compact mass detectors such as the ACQUITY QDa II Mass Detector have to offer in manufacturing support labs (USP <1086>).

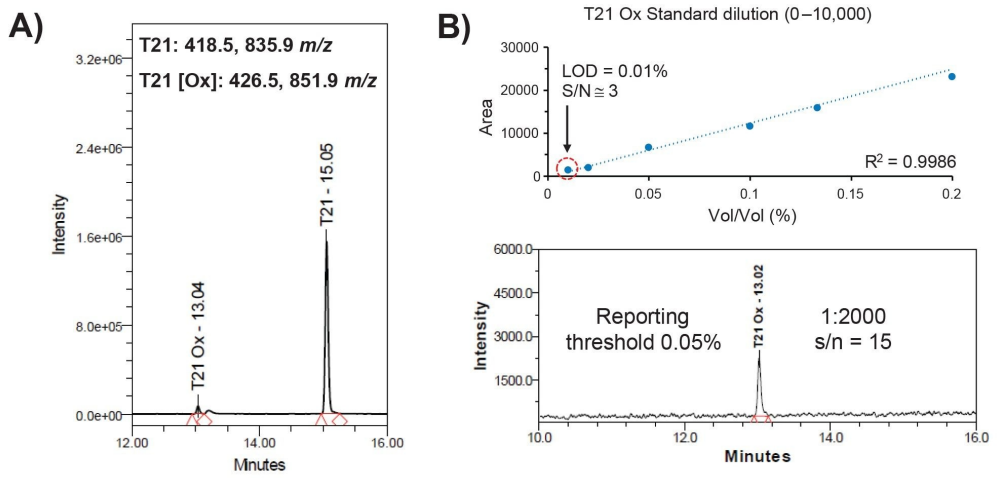


Figure 5. A) SIR chromatogram of native peptide T21 and its associated oxidized impurity T21-Ox monitored in the Waters mAb Tryptic Digestion Standard (p/n: 186009126). B) Peak area response for a serial dilution of a synthesized oxidized standard (T21 Ox) with the ACQUITY QDa II Mass Detector using SIR mode. SIR chromatogram of the normalized standard (T21 Ox) at a dilution ratio of 1:2000 representing an impurity at 0.05% (v/v).

In another aspect, the sensitivity and specificity provided by the ACQUITY QDa II Mass Detector is well suited to support formulation and stability analyses. To demonstrate this, the oxidation rate of the T21 peptide fragment was monitored over an 84-hour period to study the effects of oxygen on sample stability. Briefly, using Waters mAb Tryptic Digestion Standard, three vials of sample were prepared in an identical manner and sealed with PTFE non-slit septum caps. Twenty-four injections were made from each vial and monitored for oxidation. As shown in Figure 6, using the summary plot reporting functionality of the Empower 3 Software for visualization purposes, peak area % was plotted for the oxidized form of the T21 peptide fragment. From the summary plot, a clear trend was observed in the oxidized species over the course of the study. As shown in Figure 7A, closer inspection of monitoring runs 01, 25, and 49, which were the 1st injection from each sample vial, indicate only a 0.3% change in the oxidation level of T21 occurred in the sealed vials over 56 hours. In contrast, the same peptide fragment exhibited up to a 4.0 % change in oxidation levels over a 28-hour period following the initial puncture of the vial septum as shown in Figure 7B. This indicates that samples are at higher risk of oxidation in open-air environments and may need to be considered as part of method development conditions when monitoring oxidation levels of drug substance or products.

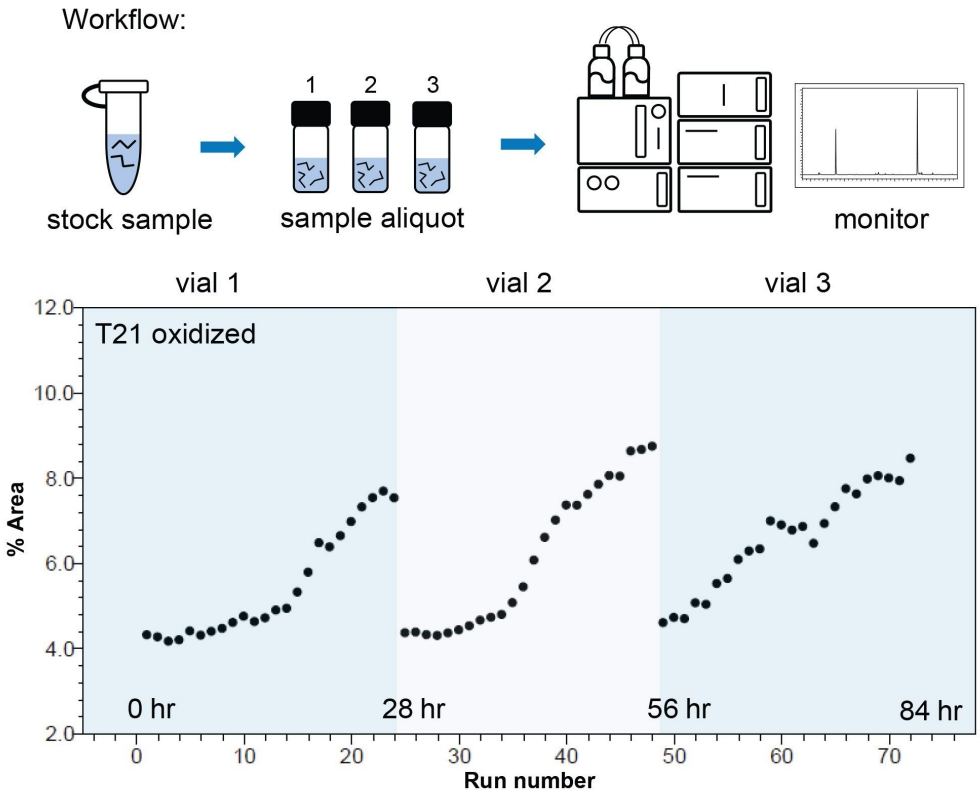


Figure 6. Empower 3 summary plot of % Peak Area for oxidized peptide fragment T21 relative to its native form over a 84-hour study using the Waters mAb Tryptic Digestion Standard (p/n: 186009126). Twenty-four injections were made from each vial in succession over the course of the study.

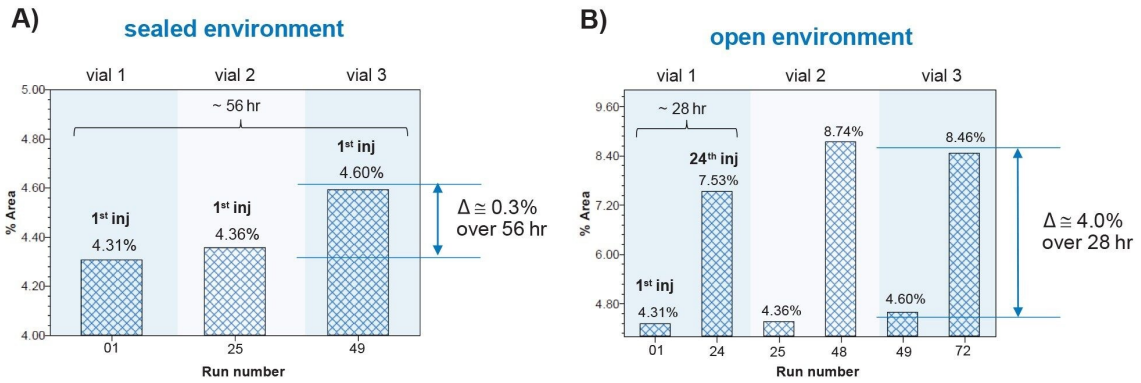


Figure 7. A) Bar plot of % Peak Area of the T21 oxidized peptide fragment relative to its native form to monitor oxidation in a A) sealed environment (1st injection from each sealed vial), and a B) open environment (1st and 24th injection from each individual vial).

As part of this time study, deamidation of the "PENNY" peptide T37 (sequence: GFYPSDIAVEWESNGQPENNYK) was monitored in parallel with the oxidized species. As shown in Figure 8A, this particular peptide is of interest as deamidation of asparagine amino acid residues in the PENNY peptide fragment is a known CQA that is present in mAb-based therapeutics and requires routine monitoring in manufacturing environments to ensure drug products are safe and efficacious.This entails ensuring methods can reliably separate and detect impurity levels in drug products for increased accuracy in impurity assessment (Figure 8B). As a pH dependent process, the deamidation impurities co-monitored in this study were significantly more stable in contrast to the oxidation impurities. As shown in the summary plots for the deamidation and succinimide intermediate species (Figure 9), no observable trends related to sample preparation or exposure to air were exhibited. More importantly, the results demonstrate the ACQUITY QDa II Mass Detector can reliably detect low abundant impurities related to CQAs. In this case, % peak area for the deamidated species and the succinimide intermediate were accurately assessed with % R.S.D. ≤ 5%. Furthermore, given that this study was performed over an extended period of time without user intervention, it illustrates the ACQUITY QDa II Mass Detector is capable of supporting automated or semi-automated workflows. This enables users to lower overall operating costs and develop QC-friendly MS methods that yield consistent and robust results.

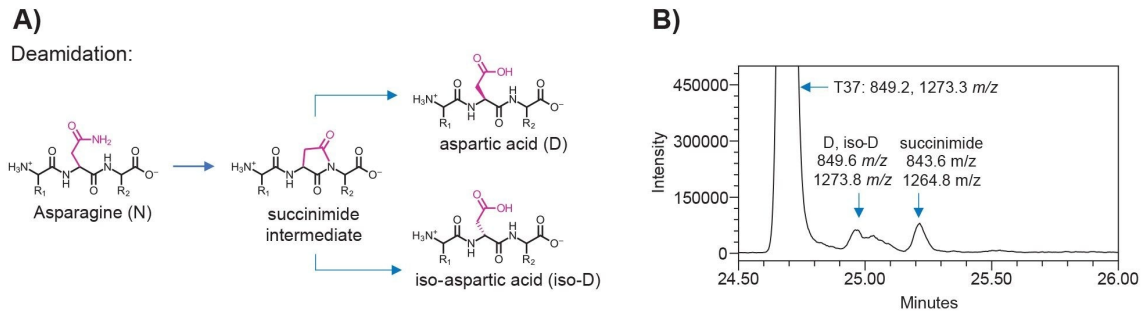


Figure 8. A) Illustration of the deamidation mechanism of asparagine in a generic peptide sequence. B) SIR Chromatogram of the T37 "PENNY" peptide and its associated deamidated species and succinimide intermediate acquired with the ACQUITY QDa II Mass Detector.

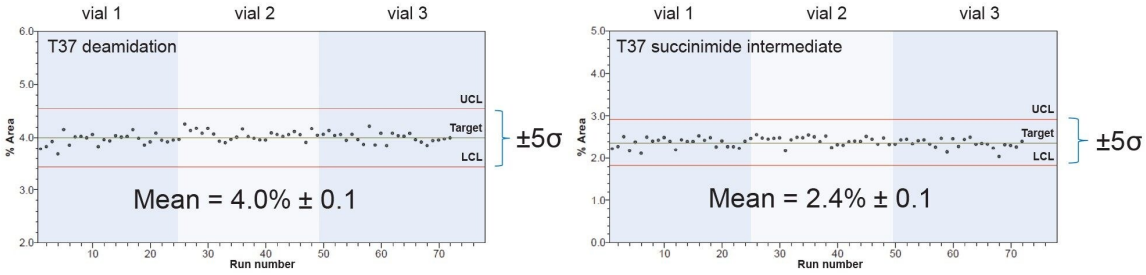


Figure 9. Summary plots of % Peak Area for the T37 deamidated species and the succinimide intermediate relative to the native T37 species. Mean % Peak Area was used as the target value and 5σ was used for upper and lower limit to evaluate data distribution.

Conclusion

- Enhanced Sensitivity for Larger Peptides: the ACQUITY QDa II Mass Detector increases the response by up to 50% for complementarity-determining region (CDR) peptides with ions above 1250 *m/z*. This improvement significantly enhances assay sensitivity, particularly for detecting larger peptides crucial in biopharmaceutical analysis.

- Detection of Low-Level Impurities: the detector identifies impurities at levels five times lower than regulatory reporting thresholds, specifically at 0.01% compared to the 0.05% standard. This capability ensures compliance with stringent USP <1086> standards, making it invaluable for maintaining product quality in manufacturing support labs.
- Cost-Effective QC Workflows: by supporting automated and semi-automated workflows, the ACQUITY QDa II Mass Detector reduces overall operating costs. It enables the development of QC-friendly mass spectrometry methods with a relative standard deviation (%RSD) of less than 5%, ensuring consistent and reliable results.
- Versatile Applications in Biopharmaceuticals: the detector's enhanced sensitivity and specificity make it effective for various applications, including identity testing of raw materials, impurity screening, and monitoring critical quality attributes. These capabilities are essential for ensuring the safety and efficacy of biopharmaceutical products.
- Productivity and Integration with Empower 3 Software: the ACQUITY QDa II Mass Detector integrates seamlessly with Empower 3 Software, supporting automated workflows that improve productivity. This integration makes it a cost-effective solution for manufacturing support labs, contributing to reduced operating costs and enhanced efficiency in drug product development and quality control.

References

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3. Dong, Q., Liang, Y., Yan, X., Markey, S. P., Mirokhin, Y. A., Tchekhovskoi, D. V., Bukhari, T. H.,Stein, S. E. (2018). The Nistmab Tryptic Peptide Spectral Library for Monoclonal Antibody Characterization. *mAbs*, 10(3), 354–369.

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