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Application News

No. B92

Quality Control Analysis of Synthetic Peptides Using the MALDI-8020 Benchtop Linear MALDI-TOF Mass Spectrometer and QC Reporter Software

Within the manufacturing process of bio-therapeutics, quality control (QC) plays a fundamental role in guaranteeing the supply of a high quality product. Any changes in the product formulation or degradation can affect the therapeutic role, leading to a potential loss of activity or development of toxicity. Amongst the several analytical techniques that can be used to determine the quality of a synthetic bio-product, MALDI-TOF mass spectrometry is widely employed due to its rapid and simple operation, low running costs, sensitivity and ability to provide information on the molecular weight as well as the sequence and structure of a compound, its impurities/adducts and modification products.

An example of a modification that can occur as result of degradation is the oxidation of methionine residues of peptides or proteins as this amino acid is highly susceptible to oxidation.

Here, we provide a complete workflow solution for the high throughput, automated quality control analysis of synthetic products using the Shimadzu MALDI-8020 benchtop linear MALDI-TOF mass spectrometer and the QC Reporter software (Fig. 1).

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Samples and Method

Four samples of synthetic peptides with different amounts of impurities were kindly provided by Bachem (United Kingdom). Exendin-4 peptide was purchased from Sigma-Aldrich. The oxidation of methionine was performed using 1 % hydrogen peroxide at 37 ºC (15 min). Following oxidation, the sample was purified using a ZipTip® C_{18} microcolumn (Millipore). For the MALDI analysis, all samples were spotted with alpha-cyano-4-hydroxycinnamic acid (CHCA, 5 mg/mL, 1:1 acetonitrile/0.1 % trifluoroacetic acid) onto the target.

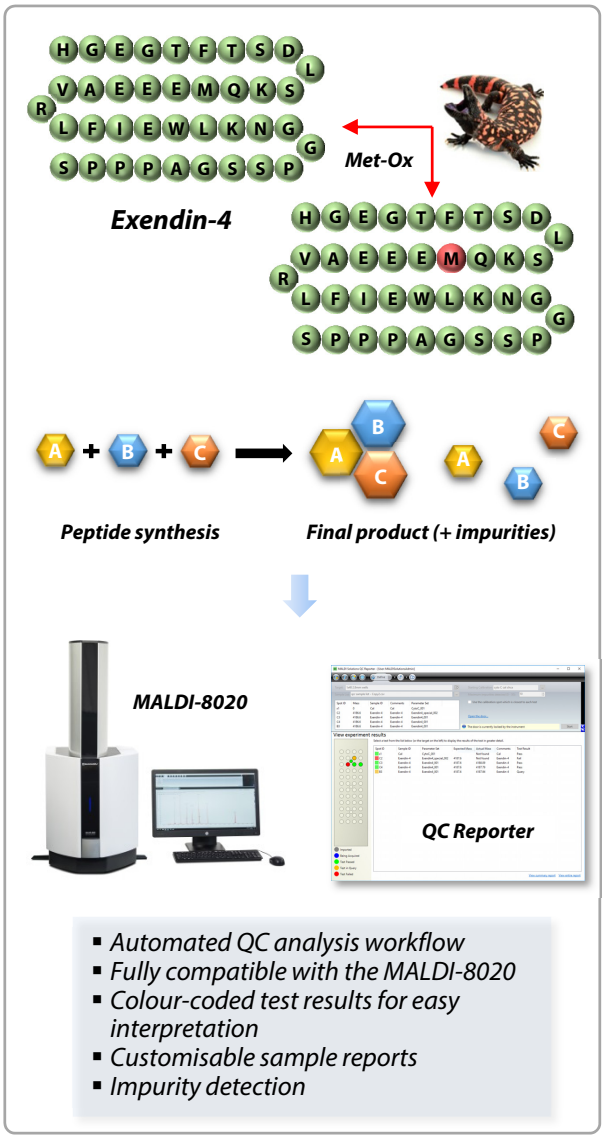


Fig. 1 Workflow Solution for the QC Analysis of Synthetic Peptides.

Results

Exendin-4 is a naturally occurring peptide present in the saliva of the Gila monster (*Heloderma suspectum*, Fig. 1). The synthetic form, Exenatide, is used in the treatment of diabetes mellitus type 2.

The analysis of Exendin-4 and its modified form was performed using the QC Reporter software to represent a typical routine quality control assay. To simulate the degradation of Exendin-4, the peptide was subjected to chemical oxidation, during which modification of the single methionine residue to its sulfoxide form occurs, and a mass shift of the native species of +16 Da is typically observed. To simulate a partial degradation, a mixture of native and met-Ox Exendin-4 was prepared.

Fig. 2 illustrates the main results window of the QC Reporter software which shows the results of the QC experiment in a colour-coded manner. The 'target' mass was set to 4186.6 Da, which corresponds to the neutral average mass of native Exendin-4. This is used by the QC Reporter software to calculate the protonated ($[M+H]^+$) form of the target species, which will then be searched in the acquired mass spectrum. The bottom-right inset in Fig. 2 shows the user-determined quality criteria settings used to calculate and generate the sample results. These refer to: mass tolerance for the target mass, maximum allowed adducts and impurity levels (set to be equal to 50 % of the area of the target mass).

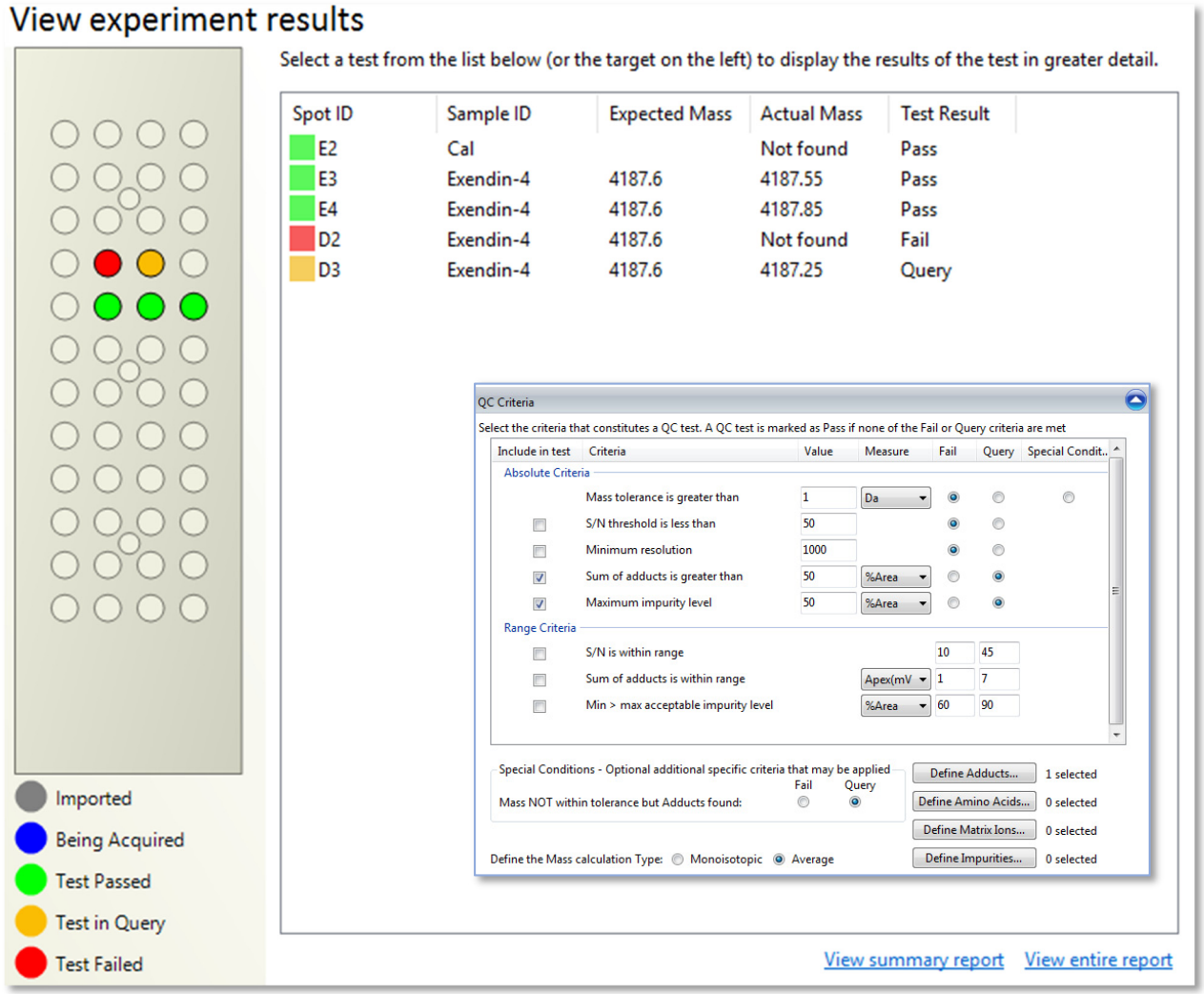


Fig. 2 QC Reporter Main Software Window Showing the Colour-Coded Results Relative to the QC Experiment of Exendin-4. The Bottom-Right Inset Shows the Quality Criteria Used to Calculate the results.

In Fig. 3a, the expected target mass (*m/z* 4187.6, $[M+H]^+$) was successfully detected and the sample (spot E3 in Fig. 2) was reported as a 'Pass'. The inset in Fig. 3a shows the part of the generated report with the details of the sample result. In Fig. 3b, the peak at the expected mass of Exendin-4 was not found, instead the oxidised species of the peptide (+16 Da; *m/z* 4203.6, $[M+H]^+$) was detected and reported (Fig. 3c). As a result, the sample (spot D2 in Fig. 2) was reported as a 'Fail'. The example shown in Fig. 3b could depict a scenario where a complete degradation by oxidation has occurred.

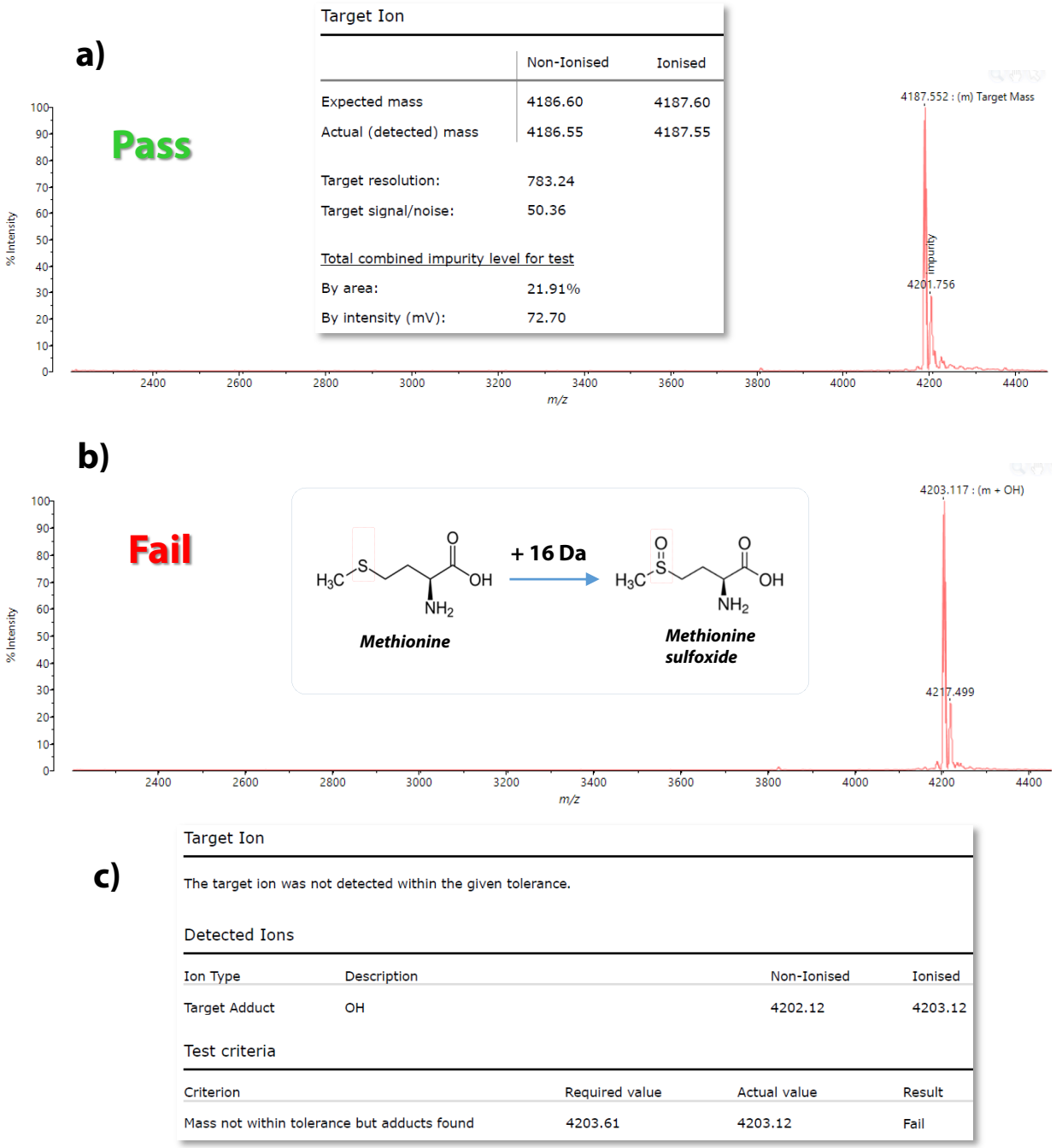
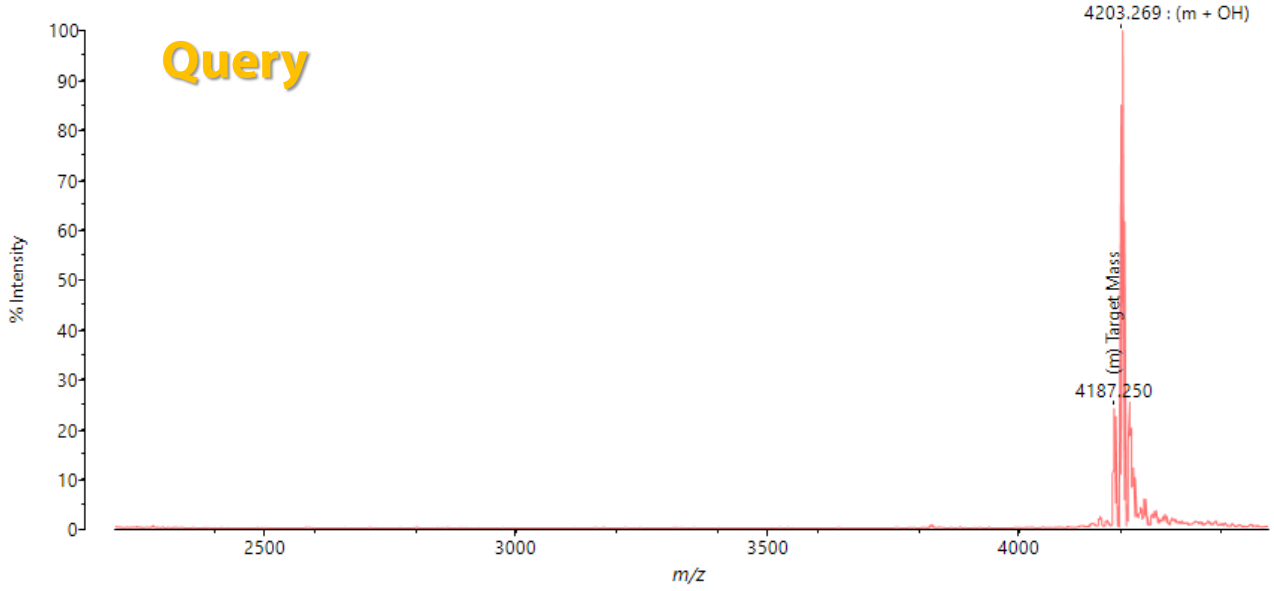


Fig. 3 a) MALDI Spectrum from the QC Experiment with Successful Detection of the Target *m/z* (4187.6, $[M+H]^+$), Which Resulted in a 'Pass' Result; Inset: Details of Sample Results from the Generated Report. b) MALDI Spectrum from the QC Experiment Which, Due to the Absence of the Expected Target *m/z*, Resulted in a 'Fail' Result; Inset: Mechanism of Oxidation of a Methionine Residue. c) Details of Sample Results from the Generated Report Highlighting that the Target Adduct (*m/z* 4203.61, $[M+H]^+$) was Detected. To Calculate the Protonated ($[M+H]^+$) Oxidised Peptide Species, OH was Specified as the Adduct, Where 'O' Corresponds to the Addition of the Oxygen Element and 'H' Represents the Ionising Proton.

In Fig. 4a, the result of the QC experiment for the native and met-Ox Exendin-4 mixture was reported as a 'Query'. This indicates that although the target mass of the native species (*m/z* 4187.6, $[M+H]^+$) was detected, the prominent peak of the met-Ox species (*m/z* 4203.61, $[M+H]^+$) exceeded 50 % of the area of the target mass, as specified in the QC criteria and reported in the sample report (Fig. 2 and Fig. 4b, respectively).

a)



b)

Detected Ions

Ion Type	Description	Non-Ionised	Ionised
Target Adduct	OH	4202.27	4203.27

Impurities detected

Non-Ionised	Ionised	Percent of target by area	Percent of target by intensity
4216.80	4217.80	146.88%	105.38%
2100.95	2101.95	10.78%	34.80%
4246.25	4247.25	5.39%	24.45%

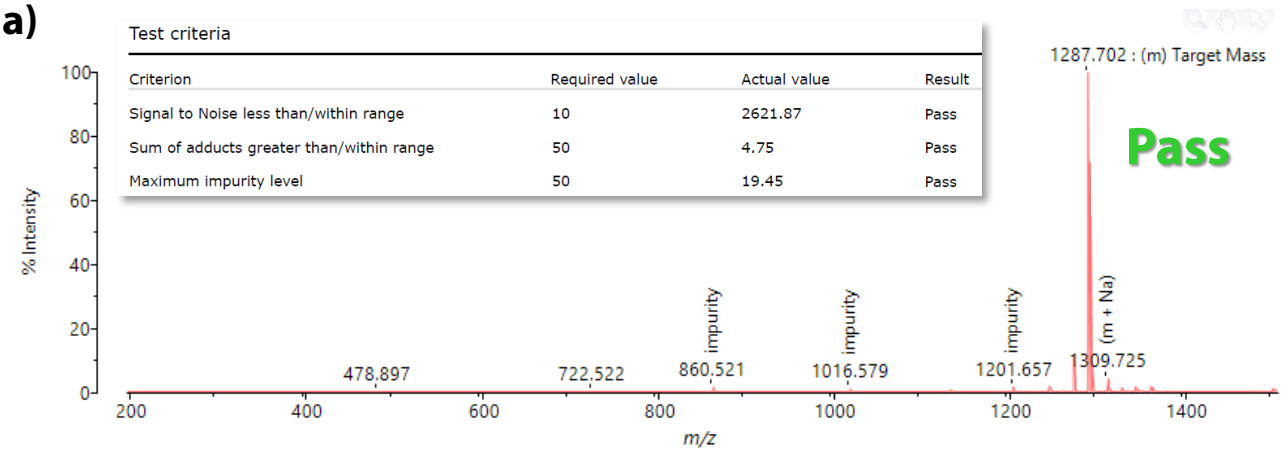
Test criteria

Criterion	Required value	Actual value	Result
Sum of adducts greater than/within range	50	557.45	Query
Maximum impurity level	50	163.04	Query

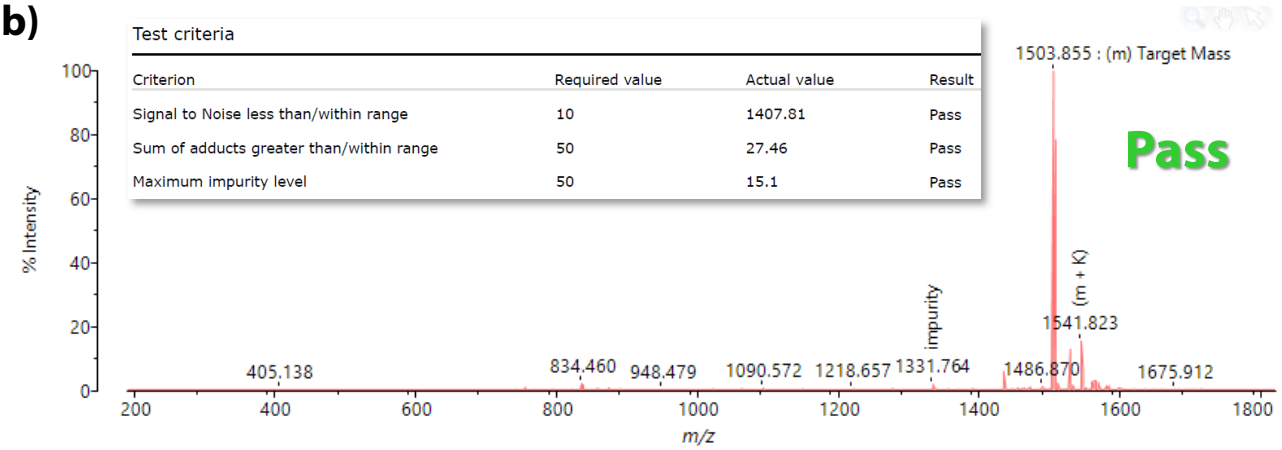
Fig. 4 a) MALDI Spectrum from the QC Experiment Which, Due to the Presence of a Prominent Peak of the Oxidised Form Concomitant to the Native Species, Resulted in a 'Query' Result.
b) Details of Sample Results from the Generated Report Highlighting that Target Adduct Amount Exceeded the Specified 50 % of the Area of the Target Mass.

Figs. 5a-c show the results of the QC experiment for three peptide synthesis products. As it can be observed, all target masses expected (*m/z* 1287.73, 1503.82 and 1740.94, $[M+H]^+$) were successfully detected. The insets in the mass spectra, from the generated sample reports, show that all three peptides achieved the QC criteria specified: minimum signal-to-noise of the target mass (set as 10), maximum amount of adducts (sodium and potassium) and of impurities, both set to be ≤ 50 % of the area of the target mass.

a)



b)



c)

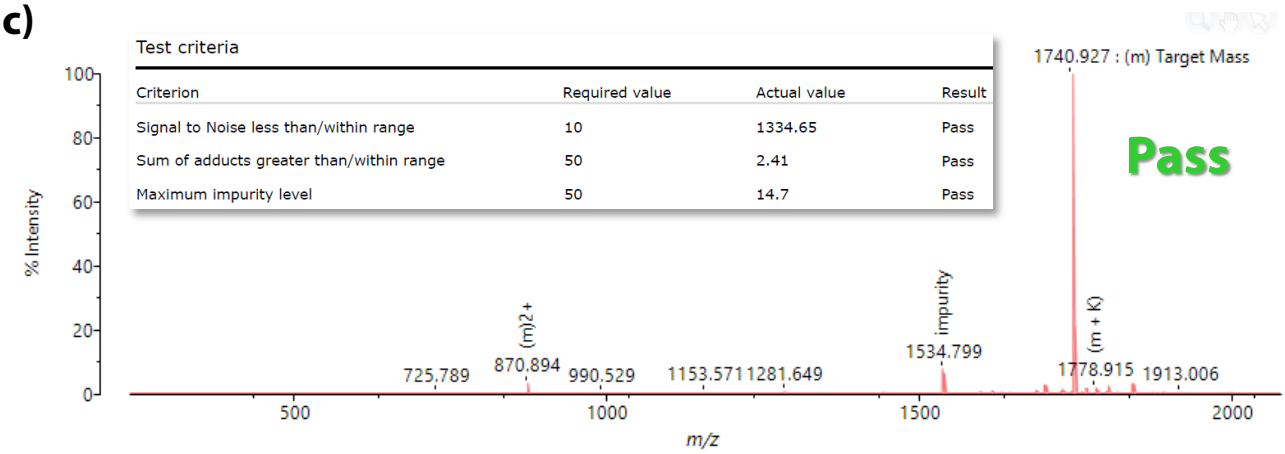
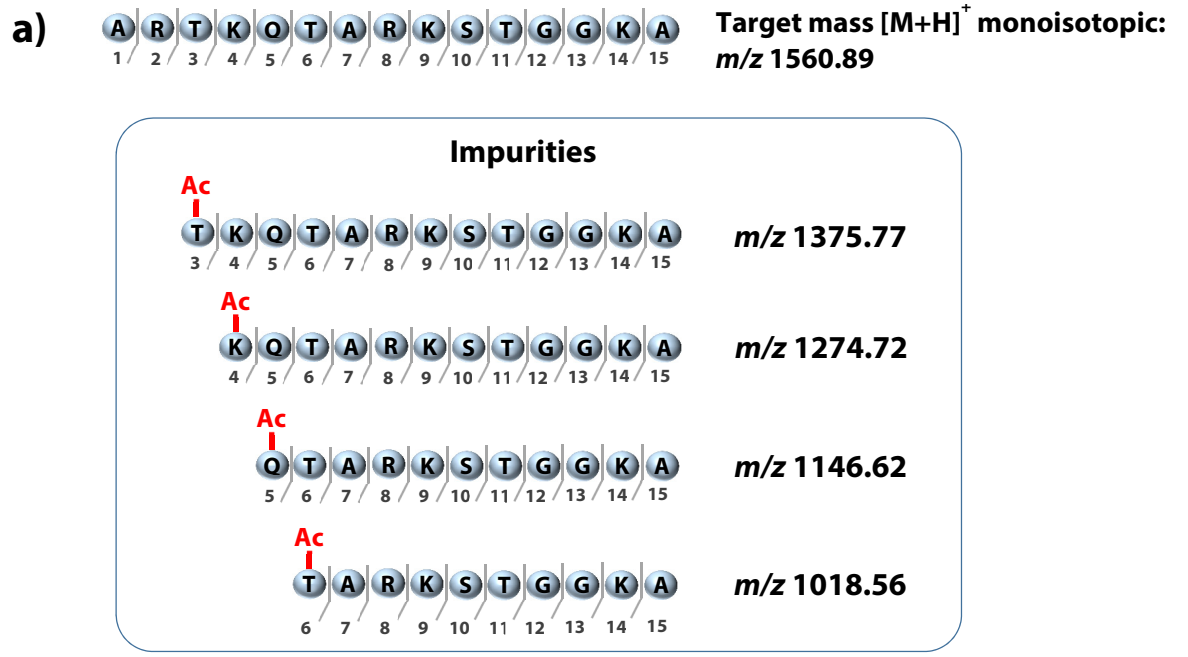
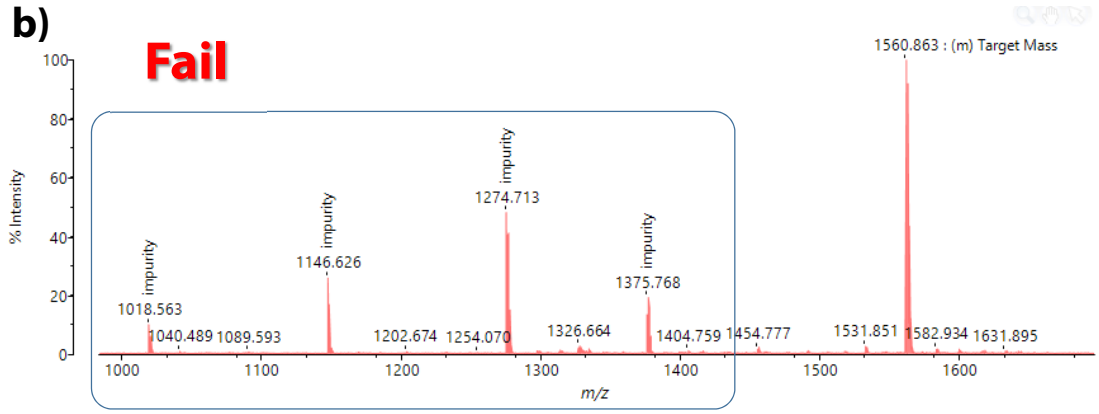


Fig. 5a-c MALDI Spectra from the QC Experiment with Successful Detection of the Target Masses (*m/z* 1287.73, 1503.82 and 1740.94, $[M+H]^+$), Which Resulted in 'Pass' Results. Insets: Details of Sample Results from the Generated Reports Highlighting that all QC Criteria Specified were Successfully Achieved.

Figs. 6a-c show the results of the QC experiment for one of the synthetic peptides, which contains significant levels of impurities consisting of truncated intermediates of the synthesis carrying an acetyl group (MWs estimated in Fig. 6a). As can be seen in Fig. 6b, the target mass (*m/z* 1560.89, $[M+H]^+$) was successfully detected along with peaks consistent with the calculated impurities. Fig. 6c illustrates the part of the generated sample report where it is shown that, due to the impurity level criterion which was not achieved, the experiment resulted in a 'Fail'.





c)

Test criteria

Criterion	Required value	Actual value	Result
Signal to Noise less than/within range	10	791.05	Pass
Sum of adducts greater than/within range	50	1.55	Pass
Maximum impurity level	50	100.53	Fail

Fig. 6 a) Sequences and Calculated Molecular Weights of Final Peptide Product and Its Impurities (Truncated Acetylated Intermediates). b) MALDI Spectrum from the QC Experiment of the Synthetic Peptide Expected at *m/z* 1560.89 ($[M+H]^+$) Which, Due to the Presence of Prominent Impurity Peaks Accounting for Greater than 50 % of the Area of the Target Mass, Resulted in a 'Fail' Result. c) Details of Sample Results from the Generated Report Highlighting that the Amount of Impurities Exceeded the Specified 50 % of the Area of the Target Mass.

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