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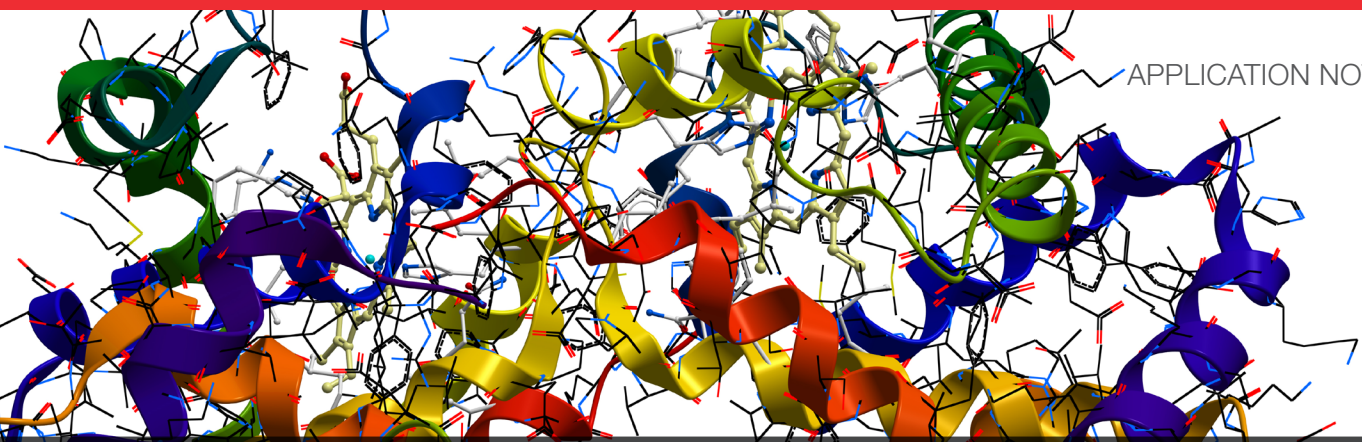

APPLICATION NOTE 21688

Robust and reproducible peptide mapping and intact mass analysis workflows on a single instrument platform

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Keywords

NIBRT, Biopharmaceutical, QA/QC, Critical quality attribute, Peptide mapping, Somatotropin, MAbPac RP, Acclaim 300 SEC, Acclaim VANQUISH C18, Magnetic SMART Digest, KingFisher Purification System, Vanquish Flex UHPLC, Q Exactive Plus MS

Application benefits

- Native and denatured intact protein analysis and peptide mapping on a single platform
- The entire workflow for the peptide mapping assay, including the digestion step, was completed in a little over one hour with minimal effort from the user
- The accurate and reproducible confirmation of the sequence with 100% sequence coverage, verification of the correct disulfide bond linkages, and quantification of several post-translational modifications
- Associated ease-of-use through automation

Goal

To demonstrate the applicability of a single Thermo Scientific™ LC-MS platform for extensive characterization of biotherapeutic proteins, by peptide mapping and intact protein analysis on the recombinant protein somatotropin.

Introduction

The biopharmaceutical industry continues to develop protein-based biotherapeutics in increasing numbers. Due to their complexity and biological production by living cells, there are many attributes that need to be analyzed to guarantee their safety and efficacy. This can involve multiple analytical

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techniques based on several different instrument platforms. There is an industry desire to simplify the processes, produce multi-attribute methodologies, and increase reproducibility between laboratories.[1] Here we use a single instrument platform and software with multiple characterization workflows that generate data for multiple quality controlled attributes.

Peptide mapping is one of the most important assays in the characterization of any biotherapeutic protein. The analysis is used to confirm that the correct sequence has been expressed for the protein and as a check for several post-translational modifications. High-resolution mass spectrometry is coupled to chromatography for peak identification and quantitation. Many QC methods use solely UV detection after the peak identities have been confirmed to simplify the method for a QC environment. However, UV-only data limits the attributes that can be measured and does not give absolute positive identification of the peptides. Here we show the possibility to use a simple, easy-to-implement LC-MS method with evidence of the benefits of such a system over UV-only detection.

Trypsin is the most commonly used proteolytic digestion enzyme due to its high specificity. However, many trypsin-based protocols and kits that have been developed for proteolytic digestion of proteins are labor intensive, prone to manual errors and may also introduce post-translational modifications during digestion.[2,3] Reproducible digestion is imperative for peptide mapping sample preparation, yet often leads to difficulty during method transfer. The digestion method used here is simplified and improved using immobilized heat stable trypsin.[4,5]

Intact protein analysis confirms that product with the correct molecular weight has been expressed and is an important characterization step for biotherapeutic proteins. High-resolution, accurate-mass (HRAM) Orbitrap mass spectrometry has been shown to be essential for this technique.[6] The same instrument platform, incorporating an easily automated change of columns and separation gradient, was used for the peptide mapping analysis. The intact molecular weight analysis was performed under native and denaturing conditions at high resolution to give isotopically resolved mass spectra.

Somatotropin is a small recombinant biotherapeutic protein used here as a model protein to describe the use of a new improved single instrument platform for extensive protein characterization analysis.

It is essential to detect, characterize, and quantify any undesirable modifications and confirm the correct product identity of recombinant proteins. In this application note, we demonstrate that typical protocols used for this type of characterization can be simplified and made more reproducible with new workflows performed on the same system. Peptide mapping is the most common analytical method employed for this purpose and delivers a wealth of information from correct amino acid sequence to the presence, location, and quantification of several post-translational modifications. Multiple quality attributes can be defined by peptide mapping analysis. Although a widely accepted and powerful technique, the digestion protocols for sample preparation are labor intensive and prone to manual errors and unwanted modifications. This can affect the quality of the analytical data and creates a source of irreproducibility. Incomplete digestion may render the accurate quantification of modifications impossible, however, a small amount of missed cleavage may enable 100% sequence coverage. A careful balance of digestion completion needs to be maintained in a very reproducible manner.

The Thermo Scientific™ SMART Digest™ kit was used for the sample preparation for peptide mapping analysis. This protocol greatly simplifies the digestion process and increases reproducibility. Intact protein analysis is a complimentary technique used to ensure the correct molecular weight of the protein biotherapeutic. Both these techniques can be performed on the same analytical platform with no change in the eluents used for chromatography. The Thermo Scientific™ BioPharma Finder™ software combines the identification and quantitation tools for peptide mapping with the deconvolution software used for intact protein analysis. Use of the described workflows on a single platform for this extensive characterization easily facilitates implementation in the laboratory.

Experimental

A Thermo Scientific™ Vanquish™ Flex UHPLC system connected to a Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer equipped with the BioPharma option was the LC-MS platform used for the analysis. The same system was utilized for the intact protein analysis, which was performed under both native and denaturing conditions with the data for all techniques analyzed using the both native and denaturing conditions. The data for all techniques was analyzed using BioPharma Finder 2.0 software.

Recommended consumables

- Deionized water, 18.2 MΩ·cm resistivity
- Fisher Scientific™ Ammonium acetate LC-MS grade (P/N A11450)
- Fisher Scientific TCEP Tris [2-carboxyethyl]phosphine (P/N 20490)
- SMART Digest Kit (P/N 60109-101)
- Fisher Scientific LC-MS grade water (P/N W/011217)
- LC-MS grade acetonitrile (P/N A/0638/17)
- Thermo Scientific™ Pierce™ formic acid LC-MS grade (P/N 28905)
- Thermo Scientific™ Acclaim™ VANQUISH™ C18 column, 2.2 µm, 2.1 × 250 mm (P/N 074812-V)
- Thermo Scientific™ MAbPac™ RP column, 4 µm, 2.1 × 100 mm (P/N 088647)
- Thermo Scientific™ Acclaim™ SEC-300 column, 5µm, 4.6 x 300 mm (P/N 079723)
- Thermo Scientific™ Virtuoso™ vial, clear 2 mL kit with septa and cap (P/N 60180-VT405)
- Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT100)

Sample pre-treatment

Somatotropin dry stock was made to a final concentration of 5 mg/mL in water with gentle swirling to aid in solubilization.

Digestion for peptide mapping

The sample was submitted to the SMART Digest protocol: 250 µg of somatotropin in 50 µL formulation buffer (5 mg/mL) was diluted to 200 µL with SMART Digest buffer. This was incubated for 5 and 15 minutes at 70 °C and 1400 rpm in an Eppendorf ThermoMixer®. The immobilized trypsin beads were then removed by spinning down in a micro-centrifuge for 5 minutes at 1,000g. The sample was gently removed and the supernatant carefully aspirated from the beads. The sample was split in half. One aliquot was reduced by adding DTT to a final concentration of 5 mM and incubated at 25 °C for 30 min. The other half remained untreated and was used for the detection of disulfide-bridged peptides and disulfide bond analysis.

Separation conditions

Instrumentation

Thermo Scientific Vanquish Flex Quaternary UHPLC system equipped with:

- System Base Vanquish Flex (P/N VF-S01-A)
- Quaternary Pump F (P/N VF-P20-A)
- Split Sampler FT (P/N VF-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Diode Array Detector HL (P/N VH-D10-A)
- Thermo Scientific™ LightPipe™ Flow Cell, Standard, 10 mm (P/N 6083.0100)
- Vanquish MS Connection Kit (P/N 6720.0405)

Mobile phase

Eluent A:	Water + 0.1% formic acid
Eluent B:	80% acetonitrile + 0.1% formic acid
Eluent C:	50 mM ammonium acetate
Flow rate:	0.3 mL/min
Column temperature:	70 °C
UV:	214 nm
Run conditions:	Tables 1, 2, and 3

Table 1. Gradient for peptide mapping with the Acclaim VANQUISH C18 column.

Retention time (min)	Flow (mL/min)	%B
0.0	0.3	4.0
30.0	0.3	70.0
31.0	0.3	100.0
34.0	0.3	100.0
35.0	0.3	4.0
60.0	0.3	4.0

Table 2. Gradient for intact protein analysis under denaturing conditions with the MAbPac RP column.

Retention time (min)	Flow (mL/min)	%B
0.0	0.3	10
0.1	0.3	10
10.0	0.3	55
10.1	0.3	90
11.0	0.3	90
11.1	0.3	10
15.0	0.3	10

Table 3. Run conditions for native intact protein analysis with the Acclaim SEC-300 column.

Flow (mL/min)	%C
0.25	100

MS conditions

Table 4. Q Exactive BioPharma MS parameter settings.

Parameter	Peptide mapping	Intact native/ denaturing conditions
Source probe	HESI II	HESI II
Polarity	Positive	Positive
Spray voltage	4.0 kV	4.0 kV
Vaporizer temp.	300 °C	320 °C/300 °C
Sheath gas flow rate [arb. units]	45	25/25
Auxiliary gas flow rate [arb. units]	12	10/5
Capillary temp.	320 °C	275 °C
Resolution (Full MS/MS^2)	60k/15k	120k/240k
Top-N MS^2	5	n.a.
S-lens RF level	50	80
Max inject time (Full MS/MS^2)	100 ms/200 ms	150 ms

Data processing

BioPharma Finder 2.0 software was used for analysis of all data acquired on the peptide and protein level. For deconvolution of isotopically resolved mass spectra of the intact proteins under native and denaturing conditions, the Xtract algorithm was used with a signal-to-noise threshold of 2, a fit factor of 80%, and a remainder of 25%.

For peptide mapping, searches were performed using a single-entry protein FASTA database with oxidation and deamidation set as variable modifications, 5 ppm mass accuracy, and a confidence level of 0.8 for MS/MS spectra.

Results and discussion

The sequence for somatotropin outlined in Figure 1 shows the expected cleavage positions for trypsin in red and the position for the disulfide bond linkages in yellow. This protein represents a good model system for peptide mapping and intact protein analysis with 18 peptides expected of varying size and two disulfide bond linkages (20 peptides when reduced). There are also several sites available for possible post-translational modifications by deamidation, isomerization, and oxidation.

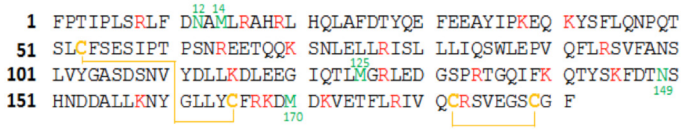

Figure 1. Amino acid sequence of somatotropin with cysteine residues involved in disulfide bonds marked in yellow and asparagines and methionine residues prone for oxidation and deamination marked in green.

Peptide Mapping

The samples obtained after performing digestion with and without subsequent reduction were subjected to LC-MS analysis under the conditions outlined above. The base peak chromatograms obtained for the reduced and unreduced samples are shown in Figure 2.

The chromatogram of the non-reduced somatotropin digest sample shows two extra peaks with retention times of 7.96 min and 16.50 min labelled with a black star in the upper panel of Figure 2. These correspond to the two disulfide-linked peptides present in the native somatotropin. The SMART Digest protocol does not use upfront reduction or alkylation as the proteins are digested in heat denaturing conditions at 70 °C. Thus, the disulfide linked peptides still maintain the covalent

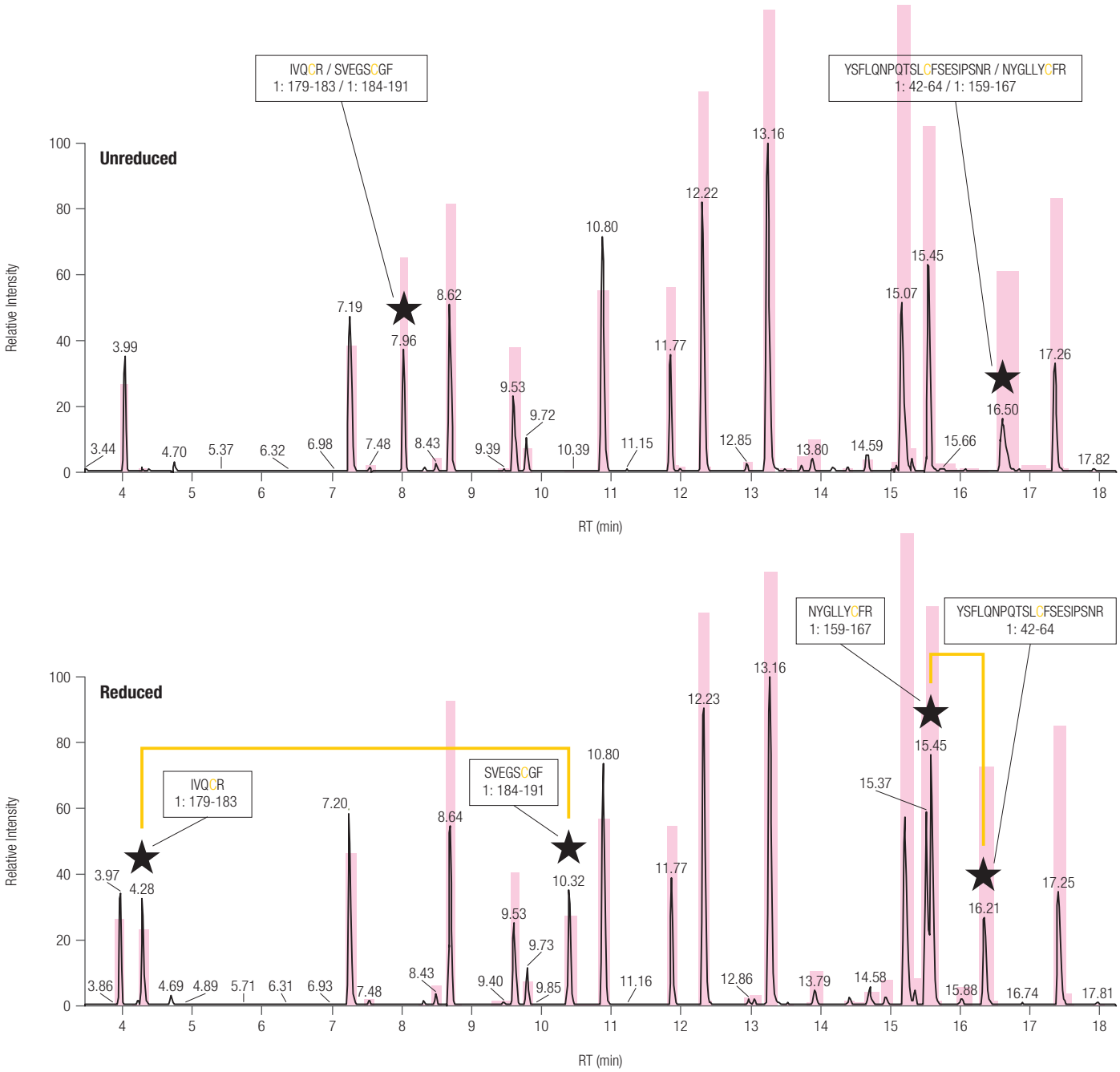

Figure 2. Base peak chromatograms obtained for the unreduced (top) and reduced (bottom) somatotropin trypsin digest. Cysteine containing peptides are labelled with black stars indicating four disconnected peptides in the reduced sample and two peptides in the unreduced sample bearing disulfide bonds.

linkage following the digestion procedure. This linkage can be broken by reduction with DTT at the peptide level following digestion and the effects are visualized in the bottom panel of Figure 2. The two peaks representing the disulfide-linked peptides in the chromatogram of the non-reduced sample disappear with the subsequent appearance of four additional peptides that are not initially observed in the non-reduced sample. The released peptides from the broken disulfide bonds are marked in the lower panel with stars. The peptides at retention times 4.28 min and 10.32 min relate to one disulfide linkage, and the peptides at retention times 15.45 min and 16.21 min relate to the other. The direct confirmation of the disulfide bond linkages is given by the BioPharma Finder software, which can identify the presence of the linked peptides in the non-reduced digestion by accurate parent ion mass alone. Further verification of the di-peptide can be achieved based on MS^2 spectra. This is an advantage of the described workflow, as an effective digestion of the unreduced protein can prove difficult to achieve with some proteins using standard in-solution digestion protocols.

The disulfide bond assignments in Figure 3 of the chromatogram are marked with a star for clarity. Unlike the other peptides that have a peak label indicating the position of the peptide in the sequence, the peak label for the disulfide-linked peptides show sequence positions for both peptides. The first to elute contains the tryptic peptides at positions 179-183 linked to the peptide at position 184-191. The second has 159-167 and 42-64 linked together by a disulfide bond. This is also shown in the sequence coverage map highlighted with yellow and red boxes, respectively. The yellow boxes indicate the peptides in the first eluting disulfide-linked peptide. The retention times indicate that they are eluting at the same retention time of 8.0 min due to the covalent linkage connecting them together. The last eluting disulfide-linked peptides are marked with a red box showing the identical elution time of 16.5 min. BioPharma Finder has this disulfide bond assignment method as a default choice from the menu. The results were obtained without any further manipulation of the

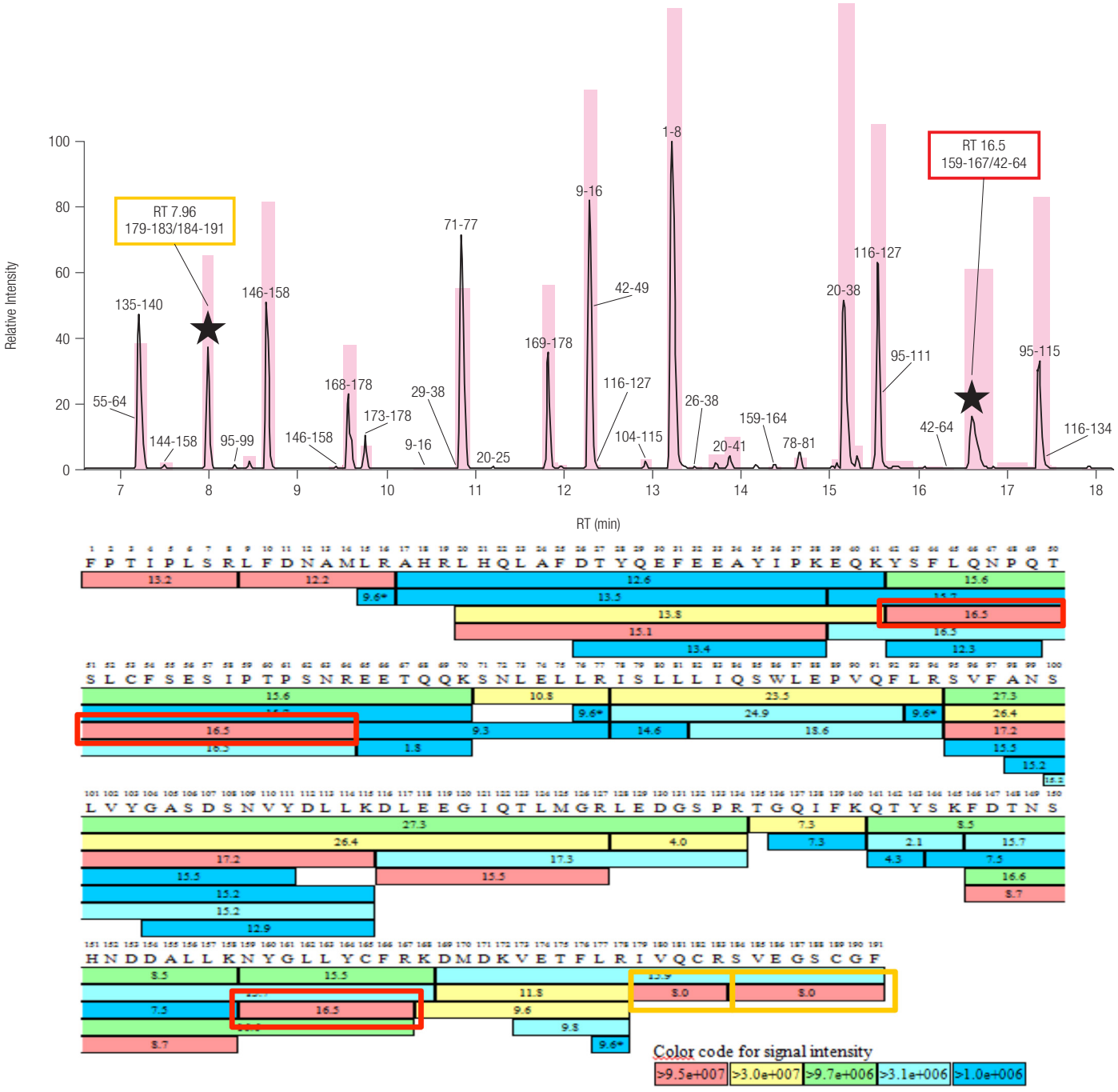

Figure 3. Assignment of peaks in the chromatogram representing peptides with intact disulfide bonds and location of the peptides involved in disulfide bonds in the protein sequence highlighted with boxes in red for the peptide aa 159-167/42-64 and with boxes in yellow for the linked aa peptides 179-183/184-191.

disulfide bond default method. Using the standard built-in methods sequence coverage of 100% was achieved for the peptide map of somatotropin without reduction of the digested peptides.

Proteins	Number of MS peaks	MS peak area	Sequence coverage	Abundance (mol)
Somatotropin	457	90.9%	100.0%	100.0%
Unidentified	14	9.1%		

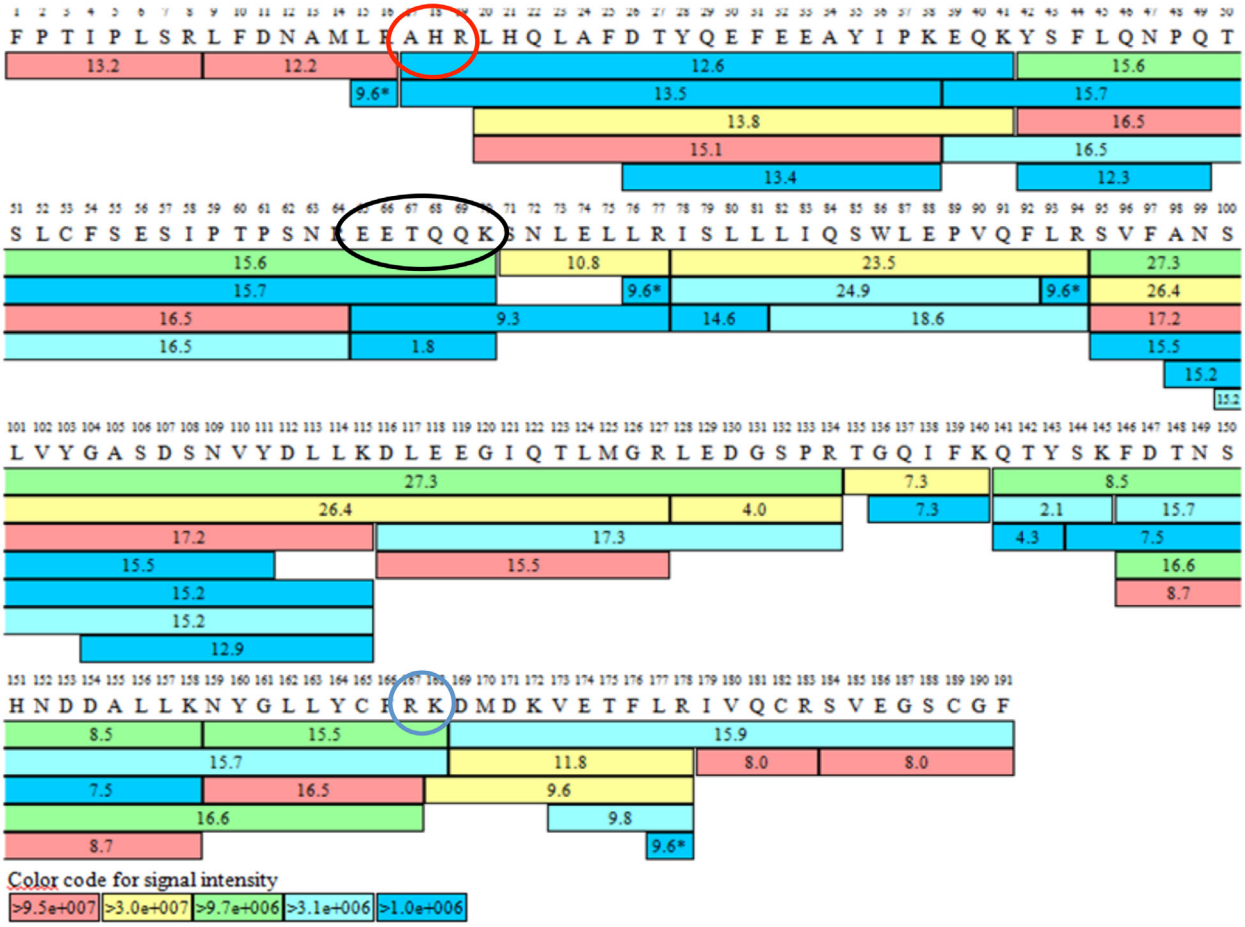

Figure 4. Sequence coverage map for somatotropin showing 100% coverage. Full MS and MS/MS data were used. The bars underneath the sequence represent peptides with colors indicating the precursor intensities and the numbers in the bars representing the retention time.

A small amount of a missed cleavage product is required to see a very hydrophilic peptide EETQQK, which is circled in black in Figure 4. The full cleavage product is difficult to see without a high sensitivity MS system and impossible with UV detection only, as it does not ionize well and elutes in the column void at a retention time of 1.8 min (Figure 4). Another short peptide is circled in red with the sequence AHR, which is also identified by a very low abundant missed cleavage product. The full cleavage product elutes very early in the column void at 1.3 min but does not ionize well at all (Figure 4). If the digestion time is increased to 60 minutes, full digestion occurs at this site and the low level missed cleavage product used in the confirmation of the AHR peptide sequence is lost (data not shown). Somatotropin also has two trypsin cleavage sites adjacent to each other at positions R167 and K168. This is circled in blue in Figure 4 with the two resulting abundant peptides eluting at 9.6 min and 11.8 min (yellow bars). Both peptides contain an unusually abundant missed cleavage site at position K172, a position just after the adjacent R167 and K168 sites and in front of a methionine at M170, which is susceptible to oxidation (Table 5). This missed cleavage is possibly due to the acidic nature of the glutamate and

aspartate residues near the cleavage site. The other trypsin cleavage sites throughout the somatotropin sequence are cleaved perfectly using the SMART Digest kit, showing high abundant peptides that are easily identified with BioPharma Finder software and can be converted to a method using UV absorption only for detection.

An additional benefit of the LCMS methodology is the identification of post-translational modifications. There are several sites in the sequence that could be prone to deamidation, oxidation, and isomerization of aspartic acid. These can be identified in BioPharma Finder software by simply adding the modifications that are interesting from a built-in list to the sequence manager. In this case, we looked for all the above modifications in the somatotropin sample. Results are indicated in Table 5 for a 5 and 15 minute trypsin digest using the SMART Digest kit.

Table 5. Post-translational modifications identified and quantified from the somatotropin SMART Digest samples after 5 min and 15 min digestion time.

Position	Sequence	Retention time [mod./unmod.]	Modification	% (5 min digest)	% (15 min digest)
N149	FDTNSHNDDALLK	9.43 / 8.68	Deamidation	1.05	1.64
M170	DMDKVETFLR	9.58 / 10.31	Oxidation	0.59	0.60
M14	LFDNAMLR	10.41 / 12.28	Oxidation	1.52	1.34
M125	DLEEGIQTLMGR	12.40 / 15.48	Oxidation	0.18	0.21
D130	LEDGSPR	3.95 / 4.06	Isomerization	0.18	0.55

There is one deamidation site, three oxidation sites, and one isomerization site identified, all at very low levels. The low levels alone would make these modifications difficult to impossible to quantify by UV only, especially in the presence of significant numbers of high abundant peptides. In addition, the isomerization site modified peptide did not separate well from the unmodified form at the peptide level with the short reversed-phase gradient chromatography used in this example. This would make their identification and quantification impossible by UV detection only with the chromatographic conditions applied. The mass differences for isomerization and deamidation modifications are also very small, which makes the use of high-resolution mass spectrometry the correct choice for confident results.

The results in Table 5 show a small increase in the levels of deamidation and isomerization modifications with time, and this has been noticed previously.[2,4] This increase is still relatively small over the digestion period with the SMART Digest Kit even if digestion times are increased to 60 minutes.[4] Optimization of the time of digestion should be considered, however, to minimize any digestion-induced modifications. For somatotropin, a 15 minute digestion seems optimal, providing a good balance between complete digestion suitable also for quantification while keeping induced modifications to a minimum.

In addition, a robust and complete digestion process is critical for the identification of modifications using high-resolution, accurate-mass (HRAM) mass spectrometry. If complete digestion is not achieved then missed cleavages around the modification site will lead to more than one peptide that contains the same modification eluting at different positions, making accurate quantification difficult or impossible. Table 6 shows the effects of a short digestion time of 5 minutes, creating a small level of an additional missed cleavage peptide for the deamidation at N149. A digestion site in front of the peptide has not been cleaved, creating an additional longer peptide with five additional amino acids, QTYSK, at the front of the sequence. In this example, the SMART Digest procedure still gives a credible result as the missed cleavage product is only present in relatively small amounts. This is made more important as the percentage levels of the deamidation product in the missed cleavage peptides are shown here to be different. An incomplete digestion would lead to several peptide products containing the same modification at different levels with the modified and unmodified peptides for deamidation all eluting at different retention times. This applies considerable pressure on obtaining an efficient reliable digestion for the assay. With less predictable and difficult to use digestion procedures this will cause a problem with accurate reproducible quantification.

Table 6. Quantification result of the deamidation level of asparagine at position 149 obtained from a sample after 5 min digestion time.
Two peptides are shown, one of which includes a missed cleavage site.

Position	Sequence	Retention time	Modification	%
Native	FDTNSHNDDALLK	8.69	None	
N149	FDTNSHNDDALLK	9.43	Deamidation	1.05
Native	QTYSKFDTNSHNDDALLK	8.48	None	
N149	QTYSKFDTNSHNDDALLK	9.11	Deamidation	1.69
			Average	0.99

In this series of experiments, the procedure using the SMART Digest kit was found to be extremely reproducible and efficient. Optimization is simple to obtain a complete digest that gives a small enough number of missed cleavages to help with sequence coverage but not enough not to interfere with the accurate determination of modifications.

The use of HRAM MS can be daunting to implement in the standard QC environment where HPLC systems with UV or fluorescence detection are the standard instruments and there is often little experience in high-end mass spectrometry. In this setting, a simple high-resolution MS-only instrument can be implemented in much the same way as another detector for the HPLC system. This adds a detector with minimal functional settings to give an additional mass trace in the chromatogram running under the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software. The additional online mass analysis enables all the advantages of quantification of some post-translational modifications as well as positive identification of the peptide sequence as demonstrated above. Figure 5 shows a peptide map of somatotropin with identification of the peptides using the intact peptide mass as measured in the MS1 scan only. Confident identification can be done in the Chromeleon CDS software, combining accurate mass and retention time with this simple system configuration. In the example in Figure 5, sequence coverage of 100% was achieved with standard settings in BioPharma Finder software. The peptides produced from the workflow give high abundant (red and yellow shading), perfectly cleaved tryptic peptides making identification easy. Missed cleavage peptides are in very low abundance (blue shading) and do not interfere with the quantitative analysis of the modifications.

Proteins	Number of MS peaks	MS peak area	Sequence coverage	Abundance (mol)
Somatotropin	205	89.6%	100.0%	100.0%
Unidentified	4	10.4%		

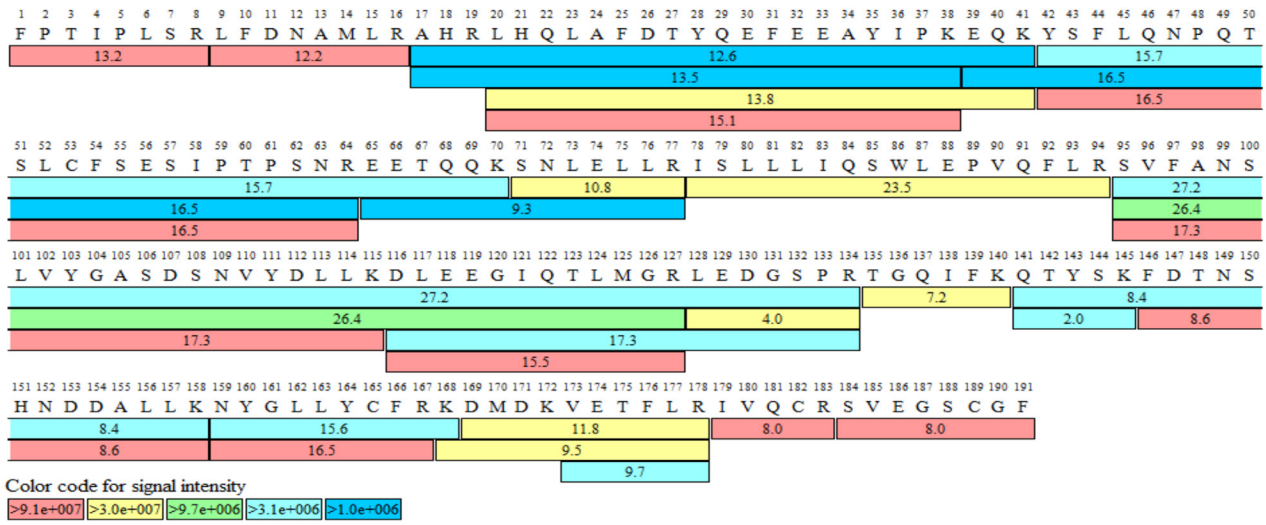

Figure 5. Sequence coverage map of somatotropin of identified peptides based on MS full scan data only.

The same system configuration can be used for denatured intact protein analysis as for the peptide mapping analysis, with a simple column change that may be performed automatically with a column change valve. The column of choice for intact mass analysis is the MAbPac RP column, a polymeric column with large pore size that exhibits high resolution of intact proteins with very little to no carryover. The eluents are the same for both analyses, with a slightly different gradient in the chosen intact analysis chromatography method. The example in Figure 6 shows the intact analysis of somatotropin with the deconvoluted monoisotopic mass at 22,111.0920 Da. The mass difference of ~4 Daltons corresponds to the four missing hydrogens lost from the cysteine residues involved in the two disulfide bonds, which were still intact in this sample. The theoretical mass with the disulfide bonds intact is 22,111.0409 Da, which is with 2.31 ppm deviation in very close agreement with the experimental value. The excellent resolution provided by the Orbitrap mass analyzer of this relatively small protein can easily be isotopically resolved at a resolution setting of 120,000. The charge envelope obtained for somatotropin analyzed under denaturing conditions spans charge states from 10 to 20 with the most abundant charge state detected at *m/z* 1,476.

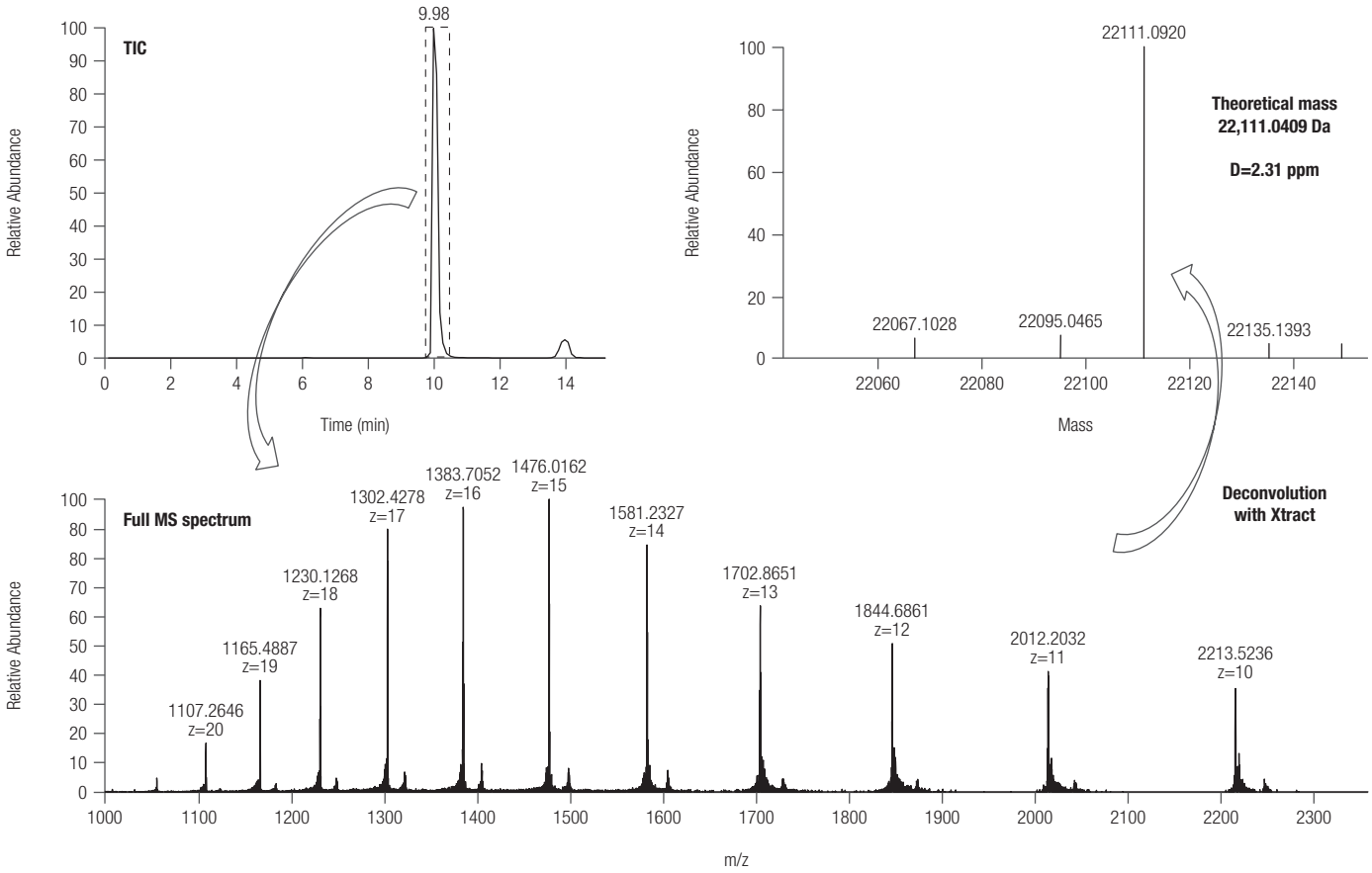

Figure 6. Mass analysis of intact somatotropin under denaturing conditions showing the total ion chromatogram, the Full MS spectrum, and the mass obtained after deconvolution with Xtract.

The mass of the native protein can also be characterized using this instrument platform. The analysis in the native form can give additional information that is not available after the protein is denatured. Binding characteristics in the native form and any higher-order structural variations can be studied with this approach. As the protein will be in its native folded state, the charges available on the surface of the protein are much lower in number than in the fully unfolded, denatured state, resulting in a reduced charge distribution on the protein and so the mass-to-charge ratio elevates to a higher *m/z* range. The reduced charge distribution can help with spectral resolution between modifications that could overlap with different variants from different charge states. For this analysis, an Acclaim SEC-300 column was utilized to introduce the intact protein to the MS, while maintaining low salt concentration. This is a polymeric size-exclusion chromatography (SEC) column that shows very little secondary interaction with proteins even at low salt eluent conditions. This was preferred over a silica-

based SEC column as it requires less salt in the eluent system to maintain excellent chromatography under size-exclusion conditions. The low salt concentration is beneficial to the mass spectrometry system for reduction of the background signal and greatly reduces the signal suppression inherent with salt gradients, resulting in overall increased sensitivity in the MS.

Figure 7 shows the base peak chromatogram, the full MS spectrum with a zoom into the most abundant charge state at *m/z* 2459.2446, and the deconvoluted spectrum for somatotropin acquired under native conditions. The distribution shows a smaller number and a shift to lower charge states due to the native form of the protein that is amenable to accepting fewer charges when compared to the analysis under denaturing conditions. The monoisotopic mass was calculated at 22,111.0754 Da, (1.56 ppm mass deviation) also in excellent agreement to the expected mass for somatotropin with the disulfide bonds intact.

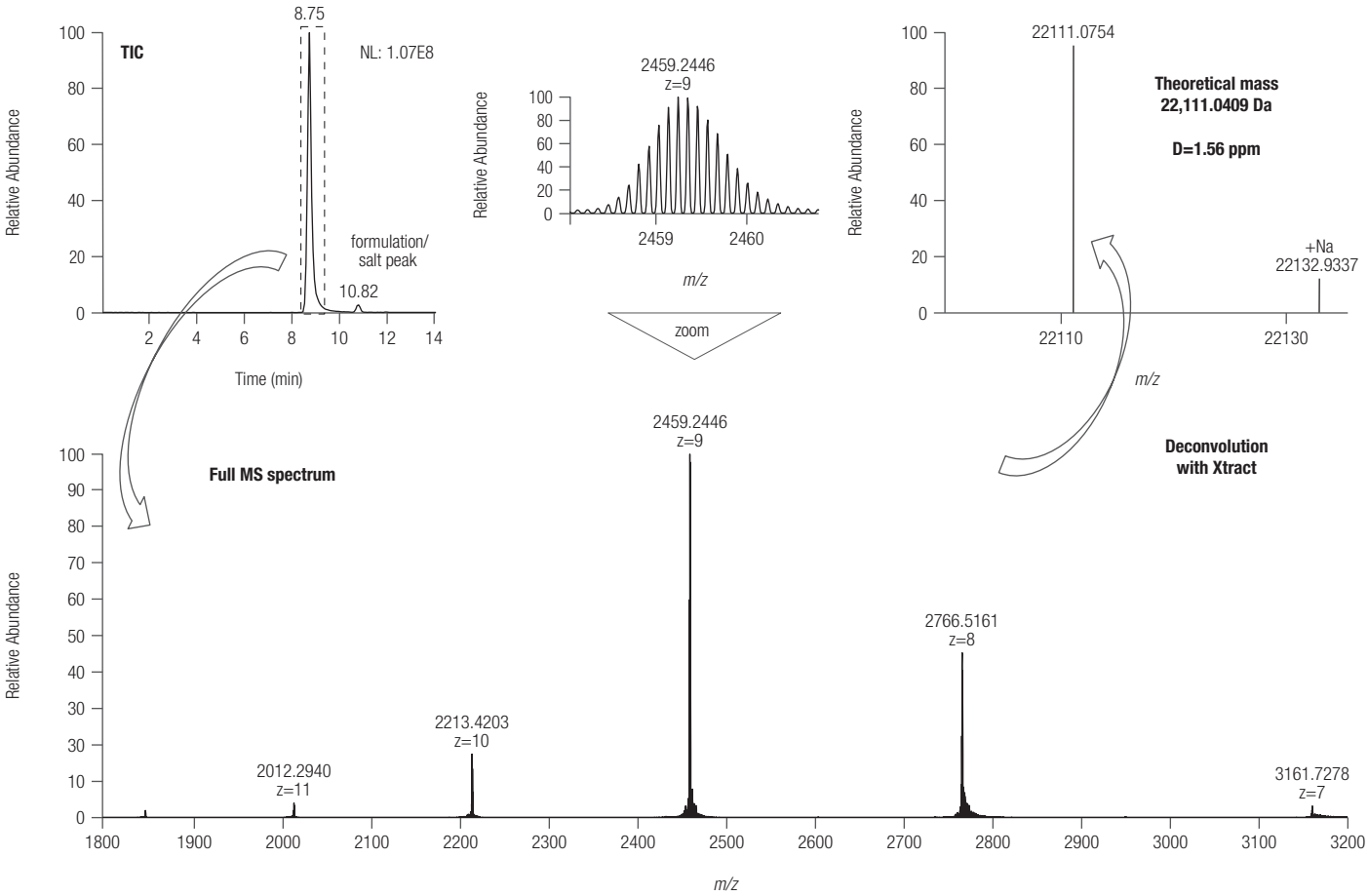

Figure 7. Analysis of intact somatotropin under native conditions showing the total ion chromatogram, the Full MS spectrum with a zoom into the most abundant charge state, and the mass obtained after deconvolution with Xtract.

Conclusions

An extensive characterization of the biotherapeutic protein somatotropin was successfully achieved using a single instrument platform. The peptide mapping workflow was simplified with excellent and reproducible data generated using the SMART Digest workflow, which showed consistent and complete digestion and good specificity for cleavage at the correct trypsin cleavage sites. This produced a simple peptide map that was easy to interpret using the BioPharma Finder software. The digestion efficiency and reproducibility was shown to be an important factor for the combination of total sequence coverage and accurate determination of posttranslational modifications. The information from the peptide mapping experiment included the following:

- Confirmation of the sequence with 100% sequence coverage
- Verification of the correct disulfide bond linkages
- Quantification of several post-translational modifications

The accuracy and reproducibility of the procedure using the SMART Digest kit allows the user to simply modify the digestion by time to allow the production of small amounts of missed cleavage products that may be required for complete sequence coverage and ensure correct conditions for complete digestion to allow accurate measurement of post-translational modifications. This is very difficult to achieve reproducibly with other digestion techniques.

The entire workflow for the peptide mapping assay, including the digestion step, could be accomplished in a little over one hour with minimal effort from the user. This assay could be implemented in a routine environment with little prior knowledge or experience in mass spectrometry using Chromeleon software to control the entire LC-MS system with the acquisition of Full MS data only.

In addition to the information from peptide mapping experiments, the verification of the correct monoisotopic mass of the intact protein based on isotopically resolved mass spectra can be done using the same system configuration. This can be achieved by analyzing the protein in the native or denatured state.

The described instrument platform and workflows show the possibilities for routine use to measure several critical quality attributes in routine characterization of biotherapeutics. The system configuration of UHPLC/MS with BioPharma Finder software has proven to be a simple, versatile, and powerful platform for the analysis of somatotropin, which could be extended to any biotherapeutic protein product.

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