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REPORT

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An evaluation of instrument types for mass spectrometry-based multi-attribute analysis of biotherapeutics

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ABSTRACT

Multi-attribute methods (MAM), based on proteolytic digestion followed by liquid chromatography-mass spectrometry analysis of proteolytic peptides, have gained substantial attention in the biopharmaceutical industry for quantifying a variety of quality attributes for therapeutic proteins. Most MAM developed so far have been based on high-resolution mass spectrometers, due to their superb resolving power to distinguish analyte signals from interferences. Lower-resolution instruments, if demonstrated suitable, may further promote the adoption of the technology due to their low cost, small footprint, and ease of use. In this work, we compared the performance of a high-resolution instrument with a few low-resolution quadrupole-type instruments in quantifying a diverse set of quality attributes in a monoclonal antibody product. Different modes of operation for the quadrupole instruments, including scan mode, selected-ion monitoring and multiple-reaction monitoring, were evaluated. The high-resolution instrument has superb performance, with a quantitation limit of 0.002%. Single-quadrupole instruments in scan mode, on the other hand, provide a quantitation limit of about 1%, which may be fit-for-purpose for many routine MAM applications.

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Multi-attribute method; quality attributes; mass spectrometry; orbitrap; single quadrupole; triple quadrupole; limit of quantitation

Introduction

Multi-attribute methods (MAM), based on proteolytic digestion and subsequent liquid chromatography-mass spectrometry (LC-MS) analysis of proteolytic peptides, are used to quantify a variety of quality attributes in protein therapeutics.[1-14] These methods take advantage of the resolving power of a mass spectrometric (MS) detector and use the MS response of each peptide isoform for quantitation. Because of the specificity of these methods toward each clinically relevant quality attribute, they have gained extensive attention in the biopharmaceutical industry.[13-17] Additionally, MAM has the advantage of monitoring a large number of attributes in a single assay, therefore potentially reducing the cost of quality by replacing several conventional methods, such as hydrophilic interaction liquid chromatography (HILIC) for glycans or cation exchange chromatography (CEX) for charge-variant analysis. Because of the universal nature of the method, a single analytical procedure can be applied to multiple products, with data processing as the only product-specific activity. As a result, the cost associated with method development and transfer is reduced. Traditionally, when a new critical product quality attribute is discovered later in the product life cycle and information on that attribute from historical batches is needed, a new analytical method must be developed to quantify the attribute with reliance on the archived samples, which may or may not be available. With MAM, on the other hand, the large amount of product quality information collected within the full-scan LC-MS data allows retrospective data processing, so no additional data need to be collected, and there is no need to rely on sample archives. Because of these advantages, MAM is increasingly used for routine product quality attribute analyses.

MS-based MAM has been performed on high-resolution mass spectrometers,[1-11] although some work has demonstrated the success of MAM on a low-resolution instrument.[12] High-resolution instruments, because of their high mass resolving power, are capable of resolving the analytes of interest from most interferences, and thereby providing superb analyte specificity. On the other hand, some low-cost, small footprint instruments are routinely used in analytical laboratories due to their ease-of-use and reliability. These lower-end mass spectrometers, if demonstrated suitable for MAM purpose, will greatly reduce the cost and footprint associated with the high-resolution instruments.

In this work, we evaluated the performance of a high-resolution instrument and several alternative low-resolution single-quadrupole and triple-quadrupole instruments, aiming at providing assessment of suitability of these instruments for MAM purpose. Different modes of operation for the quadrupole instruments were evaluated, including scan mode, selected-ion monitoring (SIM), and multiple-reaction monitoring (MRM).[18]

Results

Performance characteristics of a purity method are defined by its accuracy, precision, linearity, limit of quantitation (LOQ), and specificity (ICH Guideline Q2R1). Among these, if the

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method has acceptable specificity, precision and linearity, the accuracy of the method is guaranteed when a reference standard with known attribute abundance is used for response calibration.[8] The linearity of an LC-MS system for quantitation has been demonstrated numerous times in the past.[2,4,12,19] Other performance parameters, including precision and LOQ, can be derived from the uncertainty of measurement at different levels of attribute concentrations. Therefore, to evaluate the performance of different instrument types for MAM applications, we focused on the precision of measured abundances of a large number of attributes covering a wide range of attribute abundance (0.002% to 40%). The specificity of the method is largely determined by the chromatographic and mass spectral resolution in each set up and will also be discussed.

Table 1 lists the LC-MS systems evaluated in this work with some key MS parameters. The sample used for this study was a Chinese hamster ovary (CHO)-derived recombinant anti-streptavidin IgG2 monoclonal antibody (mAb).[3,8] For evaluation of performance characteristics of different LC-MS systems, the mAb was digested by trypsin in six replicates, and each digest was analyzed on each LC-MS system. Before the MAM analyses, the mAb digest was analyzed by LC-MS/MS four times on a QExactive orbitrap instrument (Thermo Fisher Scientific) with data-dependent MS/MS. Processing these data with MassAnalyzer (software developed in house) identified 184 attributes above the detection limit (non-zero abundances in all four runs). See Supplemental Material (Tables S1-6) for the list of these 184 attributes and determined abundances by each LC-MS setup. Clips were distinguished from nonspecific cleavages of trypsin by their increased abundance after thermal stress.[8] A workbook, containing information on retention time, peak start/end time, and accurate mass of each peptide of interest was generated from MassAnalyzer. This workbook was then further used by MassAnalyzer or MassHunter (Agilent) to quantify all attributes in other scan-mode data (both high and low-resolution). For SIM or MRM data, each peptide ion of interest is integrated using vendor-supplied software. Attribute abundance was calculated as the peak area of the modified peptide divided by the sum of peak areas of modified and unmodified peptides. Because the 184 attributes cover a wide abundance range from 0.002% to 40%, evaluating the relative standard deviation (RSD) of these attributes provides a good assessment of the measurement uncertainties at different attribute levels. The LOQ of the method for different types of attributes was defined as the minimum attribute concentration with RSD ≤ 10%.[8]

To assist understanding of instrument performance, the attributes are classified into three types.[8] Type-1 attributes are those that do not change during sample preparation/analysis (e.g., sequence variants, glycosylations, glycations, hydroxylations), type-2 attributes are those that may decrease in abundance during sample preparation/analysis (e.g., succinimide),[20,21] and type-3 attributes are those that may increase in abundance during sample preparation/analysis (e.g., oxidation, deamidation, Asp-isomerization, nonenzymatic cleavage).[4,21-24] Note that in order to consider glycosylation a type-1 attribute, the glycopeptides must not be exposed to extreme pH during sample preparation and collisional activation during ionization.[2] N-terminal-free glutamine, being a minor component compared to pyroglutamate, is considered the modified form, and therefore classified as a type-2 attribute.[8,21,25] As discussed previously,[8] instrument performance is determined by type-1 attributes because the major source of variability for type-2 and type-3 attributes often comes from sample preparation. Type-3 attributes usually have more variabilities from sample preparation because the artificial changes are based on the major unmodified form instead of the minor modified form for type-2 attributes.[8]

The six replicate digests were first analyzed by the two high-resolution LC-MS systems (A and B in Table 1), with injection of 3.0 μg of digested protein. Figure 1 shows the determined RSD of the 184 attributes on the two systems (a total of 184 × 2 = 368 data points) vs. their average determined abundances. For type-1 attributes, with very few exceptions (6 of 220), all identified attributes have RSD near or below 10%, indicating an LOQ of at least 0.002% (20 ppm). For type-2 and type-3 attributes, the LOQ is largely determined by the quality of the sample preparation procedure. For this dataset (both systems A and B), type-2 attributes exhibit LOQ about 0.01% (2 exceptions out of 41 data points) and type-3 attributes exhibit LOQ of anywhere from 0.004% to 0.3% (no exception for attributes > 0.3%), depending on the amount of variations introduced during sample preparation. Type-2 attributes include primarily N-terminal glutamines and Asn and Asp succinimides, and type-3 attributes primarily include oxidation, deamidation, Asp-isomerization, and nonenzymatic cleavages (clips). Among the type-3 attributes with RSD above 10%, most are tryptophan oxidation (Supplemental Material), suggesting more work and specific care are needed to optimize the sample preparation procedure to minimize artificial tryptophan oxidation. Counting all the 184 attributes measured on the two high-resolution LC-MS systems, the median RSD is determined to be 2.7%.

Table 1. LC-MS systems evaluated in this work.

System[a]	MS type	MS mode	Key MS parameters
A	Orbitrap	Full scan	resolution = 70 k (at m/z 200); AGC = 3E6; 2 micro scans; m/z range 300–2000
B	Orbitrap	Full scan	resolution = 70 k (at m/z 200); AGC = 3E6; 2 micro scans; m/z range 300–2000
C	Triple-quadrupole	SIM	cycle time 0.5 s; minimum dwell time 10 ms
D	Triple-quadrupole	SIM	cycle time 1.0 s; minimum dwell time 10 ms
E	Triple-quadrupole	MRM	cycle time 0.5 s; minimum dwell time 10 ms
F	Triple-quadrupole	MRM	cycle time 1.0 s; minimum dwell time 10 ms
G	Single-quadrupole	Full scan	m/z range 380–1240; scan time 0.80 s
H	Single-quadrupole	Full scan	m/z range 360–1400; scan time 0.82 s

[a]A and B have different LC models but the same MS model; C and E are the same LC-MS system in two different MS modes; D and F are the same LC-MS system in two different MS modes; G and H are different single-quadrupole models.

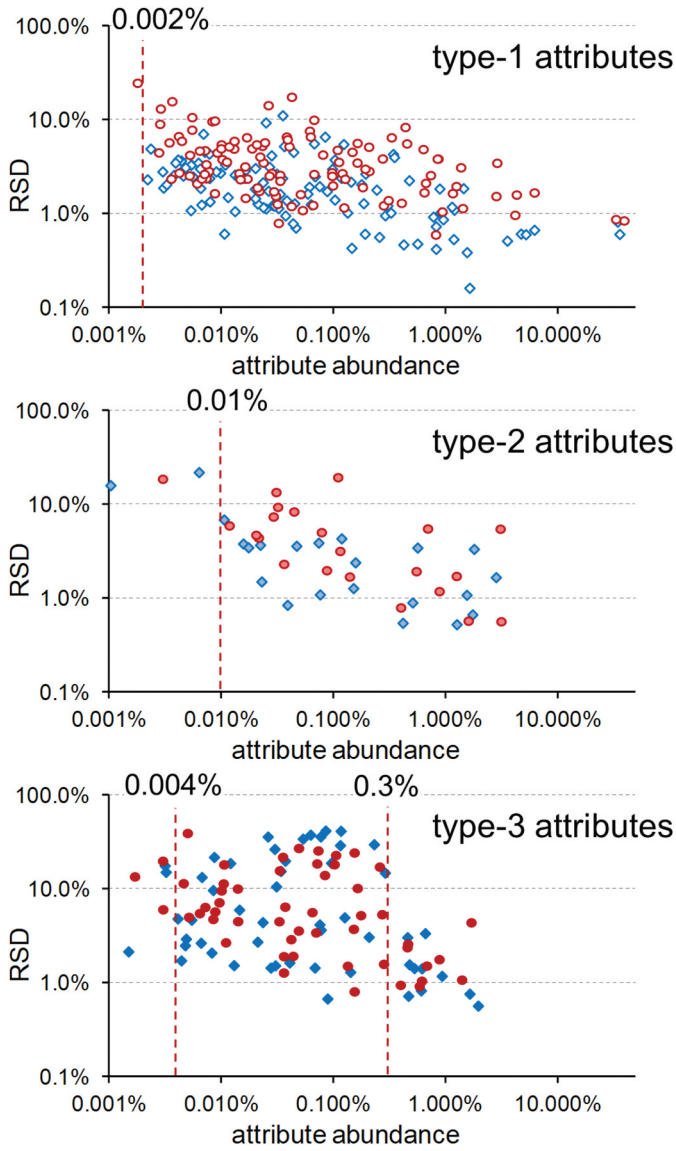

Figure 1. RSD of the six replicate measurements of the 184 attributes vs. their average abundance, as determined on the two high-resolution LC-MS systems (blue for system A and red for system B), with 3.0 µg injection of digested protein. The three types of attributes are plotted separately.

The six replicate digests were then analyzed on two triple-quadrupole instruments in SIM and MRM modes (Table 1, systems C-F). Because the performance of the method decreases with the number of monitored overlapping ions, a fraction of these 184 attributes were manually selected, so that retention time overlapping of the monitored ions was minimized. MAM, when used as a routine method, rarely needs to monitor hundreds of attributes in one experiment. Attributes monitored by the SIM and MRM experiments on each instrument are included in the Supplemental Material.

The six replicates digests were also analyzed on two low-resolution single-quadrupole LC-MS systems in scan mode (systems G and H). We expected that these systems, because of their lower mass resolution, would have difficulty resolving minor components from interferences, and therefore have higher limit of detection (LOD) and LOQ. Many minor components (abundance << 1%) were not included in the calculation because of their poor signal-to-noise ratios in the selected-ion chromatograms (SIC).

Figure 2 shows the determined RSD at different attribute abundance levels of each instrument setup compared to the same attributes determined by the high-resolution instruments. Because of the difference in instrument setup, such as m/z ranges, the charge states used for quantitation can be different. Additionally, different peptide isoforms may have different fragmentation efficiency in MRM experiments. As a result, attribute abundance determined by different LC-MS setups can be quite different. This is not a concern when a reference standard with known attribute abundance is used as a calibrant, as long as each attribute can be measured with good specificity, precision and linearity.[8] Data shown in Figure 2 indicates that quadrupole instruments in SIM or MRM modes have LOQ approaching 0.01%. Notably, the performances of Systems D and F in SIM or MRM modes approach the performance of a high-resolution instrument in full-scan mode. The performance of Systems C and E is lower because of the smaller amount of injected protein digest (0.8 µg) compared to other systems. For single-quadrupole instruments in full-scan mode (Figure 2 Systems G and H), most attributes (32 of 38) with abundance > 1% have RSD < 10% and most attributes (136 of 155) with abundance < 1% have RSD > 10%, indicating the LOQ is about 1%.

Discussion

It has been demonstrated several times that MAM results agree well with conventional methods for glycan quantitation.[2,3,7] Compared to conventional methods, however, an MS-based MAM has the inherent issue of ionization efficiency difference between different peptide isoforms, thereby affecting quantitation accuracy. This drawback is usually not a major concern, as long as the difference in ionization efficiency is consistently maintained. When the difference in ionization efficiency cannot be maintained due to an instrument change, the problem can be resolved by a run-time calibration using a well-characterized reference standard.[8]

Data obtained in this work demonstrated the power of high-resolution MS for quantitative determination of a large number of low-level product quality attributes on therapeutic proteins. Among the 184 monitored attributes in anti-streptavidin IgG2, the median RSD is 2.7% with an LOQ of at least 0.002% for attributes that do not change during sample preparation (type-1 attributes). This performance of a high-resolution instrument is also important when expanding MAM from monitoring molecular attributes to the low levels of host-cell protein impurities, where a ppm level of detection limit is often required.

Single-quadrupole instruments operating in scan mode offer a higher LOQ of about 1%. This performance, however, is comparable with many conventional methods and may be fit-for-purpose for many MAM applications, such as stability and specificity testing of common quality attributes. More importantly, because many critical quality attributes monitored in stability and specificity testing are type-3 attributes

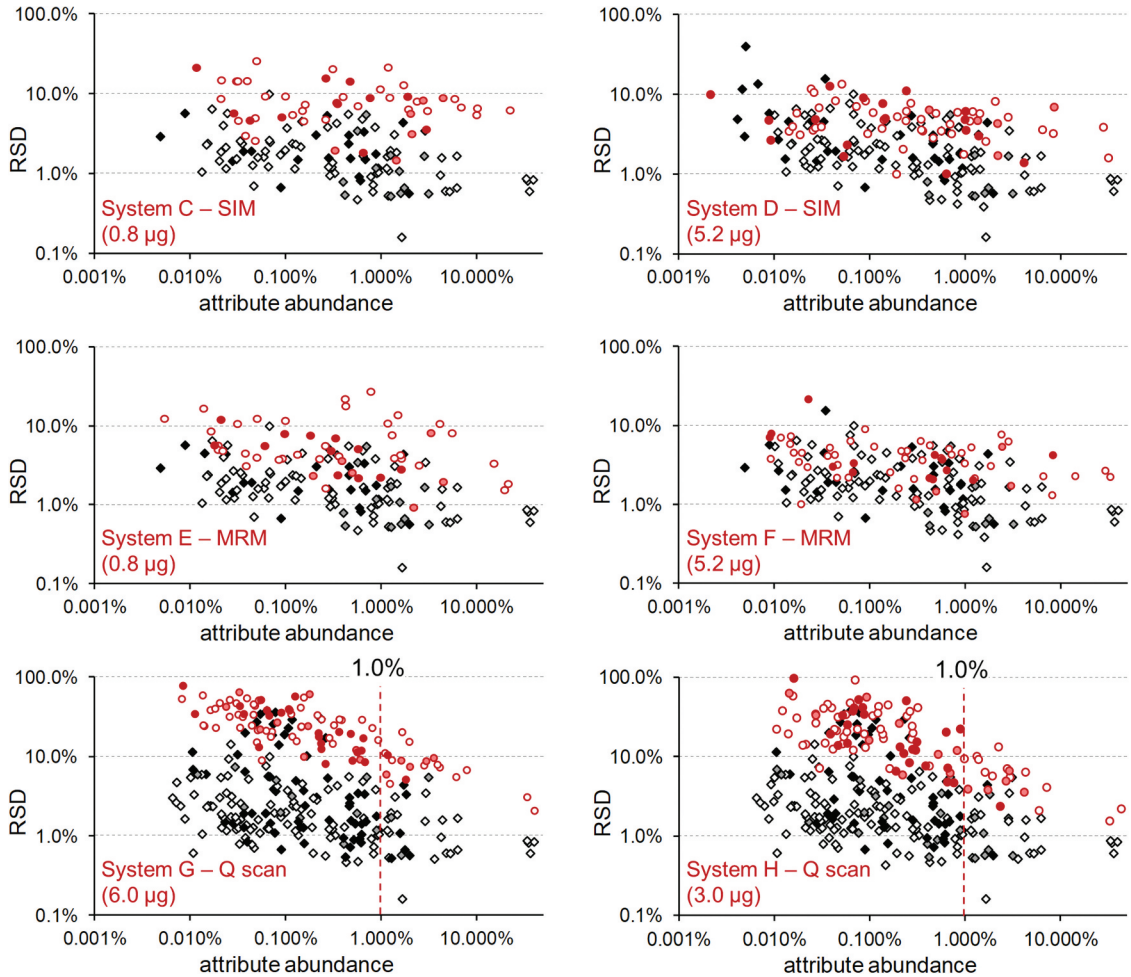

Figure 2. RSD at different attribute abundances for each quadrupole instrument setup (red circles), compared to the RSD of the same attributes determined by the high-resolution instruments (black diamonds). Open shapes are type-1 attributes, lightly filled shapes are type-2 attributes and darkly filled shapes are type-3 attributes. Injection amount is listed inside the parentheses.

(e.g., deamidation, oxidation, isomerization, clips), use of a high-resolution instrument will often not significantly improve their precision because most variabilities in measuring these attributes are from sample preparations, placing importance on optimized sample preparation conditions. Because of the limited mass resolution of single-quadrupole mass spectrometers, however, care must be exercised so that minor components are chromatographically resolved from interferences with similar m/z. Incorrectly integrating interfering peaks may produce large errors. New-peak detection may be performed on the low-resolution full-scan data, although the detection limit of new peaks may not be as low as on a high-resolution instrument. The difference is not expected to be as striking as attribute quantitation because the major source of variability in new-peak detection is from sample preparation.

As a long-term strategy, high-resolution instruments are the preferred choice for their ability to monitor a large number of attributes with very different levels. In addition, there is a trend toward the development of high-resolution instruments that are smaller, more robust and more user-friendly, and therefore potentially more suitable for routine laboratory use. At the current stage, on the other hand, single-quadrupole instruments in scan mode may be preferred in many applications when it is not critical to measure attributes below the 1% level.

Although the two targeted data collection techniques SIM and MRM performed much better than scan mode, they are less preferred choices because they require substantial efforts in developing different acquisition methods for different products. More importantly, they lack the retrospective data processing capability, which is a huge advantage of MAM when data are collected in scan mode, compared to traditional methodologies. In addition, full-scan-based MAM offers the advantage of new or missing peak detection, which is usually considered a requirement for the successful implementation of MAM in a quality control environment.[13,14,17] Compared to SIM and MRM experiments, a high-resolution full-scan configuration should be a more preferred option, when a low LOQ is required.

The LOQ of 0.002% or lower on a high-resolution instrument requires chromatographic separation of the minor components from other major components. If these components are not resolved, the LOQ is higher and largely determined by the intra-scan dynamic range of the mass spectrometers (refer to Figure S1 for an example). For the high-resolution orbitrap instrument used here (systems A and B), the intra-scan dynamic range is in the order of 1000, meaning that the minimum detectable signal of a minor component is ~0.1% of the coeluting major component, which sets the limit of the LOQ of the minor component.

Practically, the intra-scan linear dynamic range is lower than 1000 because of the requirement of accurately quantifying each of the isotope peaks with lower abundances. Assuming isotope peaks of 25% of the most abundant isotope needs to be quantified, the practical linear dynamic range becomes 250. Inter-scan dynamic range is higher than the intra-scan dynamic range because of the differences in ion injection time. In this dataset, the shortest injection time for the most abundant peptides is about 1 ms, while the longest ion injection time is 200 ms (maximum injection time setting), which further increases the dynamic range by 200 folds, making the total inter-scan dynamic range of 250 × 200 = 50000, which corresponds to an LOQ of 1/50000 = 0.002% as determined in this work. Therefore, to further increase the linear dynamic range (i.e., lower LOQ), one needs to decrease the shortest injection time by improving instrument sensitivity or increasing sample injection amount, and increase the longest injection time by applying a longer maximum injection time setting and reducing background ions. When a peptide containing a low-level critical quality attribute coelutes with another major peptide, it is advisable to optimize the chromatographic condition to resolve the two peptides.

In this study, the high-resolution instruments were set up to sacrifice some specificity for sensitivity. For example, we used a resolution of 70,000 instead of 140,000[3] for faster scan speed, and an AGC (automatic gain control) target of 3E6 instead of 1E6[3,4] for more ion counts but lower mass accuracy due to space charge. However, the observation that a low-resolution quadrupole instrument in SIM mode produced similar performance as MRM mode (Figure 2) suggests that analyte specificity is not the limiting factor in method performance. Therefore, it is potentially possible to further improve the performance of the high-resolution instrument by sacrificing more specificity (lower resolution) for a gain of sensitivity, which would be important for expanding the scope of MAM from monitoring molecular attributes to also monitoring process impurities such as host-cell proteins, either as a single method or as a separate method.

In summary, we evaluated the performance of MAM on different types of instruments using a large number of attributes with abundances varying from 0.002% to 40%. High-resolution instruments were demonstrated to have ultra-high performance, with LOQ as low as 0.002%. Single-quadrupole instruments in scan mode have an LOQ near 1%. Single-quadrupole instruments in scan mode are therefore recommended when analysis of attributes below 1% is not critical, while high-resolution instruments are recommended when higher performance is desired. Quadrupole instruments in SIM or MRM modes are not recommended due to the complexity of the method development process and the lack of new peak detection, as well as retrospective data processing capabilities.

Materials and methods

Tryptic digestion of an anti-streptavidin IgG2 MAb

A recombinant anti-streptavidin IgG2 mAb[3,8] expressed in CHO cells (prepared in-house) was used in this study. The mAb sample was digested with trypsin in six replicates using the following procedure. First, each sample was treated with 8 mM dithiothreitol (DTT, Sigma-Aldrich, St. Louise, MO) at 25°C for 30 min in a denaturing solution containing 6.4 M guanidine hydrochloride (Macron Fine Chemicals, Stroudsburg, PA), 2 mM EDTA, and 0.2 M Tris (TEKnova, Hollister, CA) at pH 7.5 to reduce the disulfide bonds. The reduced mAb sample was then alkylated with 14 mM iodoacetic acid (Sigma-Aldrich) at 25°C for 20 minutes in dark. Alkylation was quenched with 6 mM DTT. The reduced/alkylated sample (~2.6 mg/mL IgG2 concentration) was exchanged into the digestion buffer containing 0.1 M tris and 50 mM methionine (pH 7.5) using a Bio-Rad (Hercules, CA) Bio-Spin 6 column according to the manufacturer's recommended procedure. After buffer exchange, an appropriate amount of trypsin (Sigma-Aldrich) was added to achieve an enzyme:substrate ratio of 1:12, followed by incubation at 37°C for 60 min. Digestion was quenched using an equal volume of 0.25 M acetate buffer (pH 4.8) in 8 M guanidine hydrochloride. Final peptide concentration in the digest was ~0.86 mg/mL, assuming 100% peptide recovery.

Liquid chromatograph method

Each proteolytic digest of the anti-streptavidin IgG2 mAb was analyzed by reversed-phase liquid chromatography on an Agilent Zorbax RRHD Stable Bond C18 column (2.1 × 150 mm, 1.8 µm particle, 300 Å pore size) at a flow rate of 0.25 mL/min with the column temperature maintained at 50°C. Mobile phase A was 0.1% (v/v) formic acid in water, and mobile phase B was 0.1% (v/v) formic acid in acetonitrile (ACN). After initial hold at 1% B for 5 min, mobile phase B linearly increased to 10% in 1 min, then 35% in 64 minutes. Column wash was achieved by increasing mobile phase B to 90% in 2 minutes with hold for 5 min, followed by cycling the mobile phase from 1% to 90% mobile phase B in 22 min. Column was equilibrated with 1% B for 14 min. All LC-MS systems evaluated in this work used the same liquid chromatography method. The LC instrument was either an Agilent 1290-II, a Waters Acquity, or a Thermo Fisher Vanquish. Although minor drift in retention time was observed from one LC instrument to another, the drift was accounted for during data processing.

MS setup

Before MAM analyses of the six replicate digests by the LC-MS systems described in Table 1, an anti-streptavidin IgG2 mAb digest was first analyzed by LC-MS/MS four times on a QExactive Biopharma orbitrap mass spectrometer (Thermo Fisher) for peptide identification purpose. The mass spectrometer was set up to perform full-scan MS at a resolution of 70,000 and AGC target of 1E6, followed by five data-dependent higher-energy collision dissociation (HCD) MS/MS (normalized collision energy = 27%) of the most abundant ions.

For MAM analysis, the orbitrap instruments (Systems A and B) were set up to perform full-scan MS in centroid mode at a resolution of 70,000 (at m/z 200), AGC target of 3E6, and maximum injection time of 200 ms, with 2 micro-scans (Table

1). Instrument control and data collection were accomplished by Chromeleon software (Thermo Fisher).

For single-quadrupole full-scan mode experiments (systems G and H), each system was set up to collect full-scan data with the key scan parameters described in Table 1. Instrument control and data collection were accomplished by either Chromeleon (Thermo Fisher) or OpenLAB CDS ChemStation Edition (Agilent). Data were collected in profile mode.

SIM and MRM experiments were performed on the triple quadrupole instruments (systems E-H) with key parameters described in Table 1. A list of ions (m/z and retention times) to be monitored was first exported from MassAnalyzer (see next section for details) after analyzing the dataset obtained from the high-resolution LC-MS/MS experiment. Some representative ions were then manually selected from this list to minimize time overlapping between the ions. For SIM, zero collision energy was used in the pseudo-SIM mode (product ions to be the same as the precursor ions). For MRM, the top two most abundant product ions were first predicted by MassAnalyzer,[26-29] and then collision energies were optimized for maximal signal intensities of the two product ions. To maximize dwell times, peptides were monitored only in the expected elution time with a minimum of one-minute time window. Data were acquired in unit resolution.

Data processing

Custom software MassAnalyzer[1] (available in Biopharma Finder from Thermo Fisher) was used to process all scan-mode data collected by Chromeleon (orbitrap and single-quadrupole instruments). First, the LC-MS/MS data were processed by MassAnalyzer to perform peak detection,[1] retention time alignment,[30] and peptide identification. Peptide identification was accomplished by comparing the experimental MS/MS to theoretically predicted MS/MS of each candidate peptide precursor.[26-29] Peptide search space included all tryptic and semi-tryptic peptides of the IgG2 mAb, as well as commonly observed modifications and amino acid misincorporations.[31-33] A list of peptide ions to be used for attribute quantitation, including retention time, start and ending time of the peak, m/z, charge, and peptide identification information, as well as additional ions used for retention time alignment purpose, was exported as the MAM workbook. This workbook was further edited manually to remove any attributes not to be monitored. The ion list contained in this workbook was then used for detecting and quantifying all attributes in MAM data generated by the LC-MS systems described in Table 1.

To detect and quantify all attributes (specified in the workbook) in MAM data collected by Chromeleon, MassAnalyzer first performed peak detection in each raw file, then performed retention time alignment of all detected peaks to the ions in the workbook.[30] After alignment, the ions in the workbook that were not detected in the first round of peak detection were detected in the second round (gap-filling).[30] SICs were constructed using a matched window function as described previously[1] to maximize signal-to-noise ratio. For low-resolution profile data, the matched window function was constructed by modeling each isotope peak with a gaussian profile with a peak width (at half height) of 0.75 u. Integration was performed on each peak in the SIC to calculate the peak area, and then the abundance of each attribute was quantified by dividing the peak area of modified peptides by the total peak areas of modified and unmodified peptides. The robust retention time alignment algorithm used in MassAnalyzer greatly reduced the integration error. Because this program (1) applies peak-specific smoothing based on the width information of each peak provided in the workbook, (2) performs retention time alignment based on a few hundred retention-time markers provided in the workbook, and (3) identifies interfering ions as they are also present in the workbook as "dummy" ions (i.e., interfering ions that may potentially be misidentified as an analyte), it virtually eliminated the chance of false-positive peak detection for high-resolution data. For example, the MAM workbook was generated from data collected on system A. When the digests were analyzed on system B, retention time shifts from 0.16 min to 1.56 min were observed. The MAM workbook was applied to the data collected on system B, and 2112 peak detection/integration events were automatically performed. These integrations were manually checked for errors and no major integration errors (i.e., false-positive peak detection) were observed.

All other MAM data generated from single-quadrupole or triple-quadrupole instruments (scan mode, SIM, and MRM) were processed by data processing software provide by the vendors (e.g., MassHunter from Agilent). To process SIM/MRM data, ions were extracted with a left and right extraction of 0.5 u. All peak detection and integration for low-resolution data were manually verified and adjusted by comparing to the retention time profile of data collected on a high-resolution instrument.

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Disclosure of potential conflicts of interest

This work obtains and analyzes data from various MS system formats, and is for informational and educational purposes only. No endorsement or criticism of any vendor or vendor's equipment is made or intended.

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Abbreviations

MAM	multi-attribute method
LC	liquid chromatography
MS	mass spectrometry
MS/MS	tandem mass spectrometry
CHO	Chinese hamster ovary
IgG	immunoglobulin gamma
SIC	Selected-ion chromatogram
LOQ	limit of quantitation
LOD	limit of detection
RSD	relative standard deviation
DTT	dithiothreitol

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