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Use of Multi Attribute Method by mass spectrometry as a QC release and stability tool for biopharmaceuticals – Regulatory Considerations

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Position Paper

1 Introduction and background

Biopharmaceuticals require extensive quality control (QC) testing for batch release and during stability monitoring using multiple high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) based purity/impurity assays. Considering the time needed to a) develop, validate, and transfer this set of analytical methods and b) to execute them on all release and stability samples, this QC testing approach employing multiple analytical method is not supportive of accelerated product development. Moreover, the aforementioned analytical methods address categories of product-related variants (e.g., oxidized variants, charge variants) but do not always allow easy separation of individual product quality attributes (PQA) that have relevance to safety and efficacy, as these methods lack the specificity that allows location of potential chemical changes on the polypeptide backbone. Therefore, many applied purity/impurity test criteria are based on the method rather than on the specific molecular quality attribute.

Multi-Attribute Method (MAM) by mass spectrometry (MS) (referred to as MAM in this document) has been shown to overcome these limitations by allowing the quantification of multiple product quality attributes with high specificity within a single method and in a highly automated fashion by using mass spectrometry (Figure 1) ([2], [10]). MAM is an enabler of a Quality-by-Design (QbD) approach, providing enhanced product and process understanding that support the implementation of risk-based approaches to accelerate product development (ICHQ8, [1]). By assessing amino acid position-specific (called “site-specific” in this manuscript) critical quality attributes (CQA), a better-informed process parameter risk-ranking and attribute range setting can be applied across the overall control strategy including QC release and stability testing. Parallel monitoring of multiple site-specific CQAs during

stability testing is expected to improve the quality of data-driven stability modeling approaches by providing a direct link to the related degradation pathways. Overall, by allowing direct CQA measurement, MAM is envisioned to facilitate the development of a CQA-focused control strategy, at increased speed that will ultimately ensure more meaningful quality control of pharmaceutical products, potentially shortened development timelines, and earlier patient access.

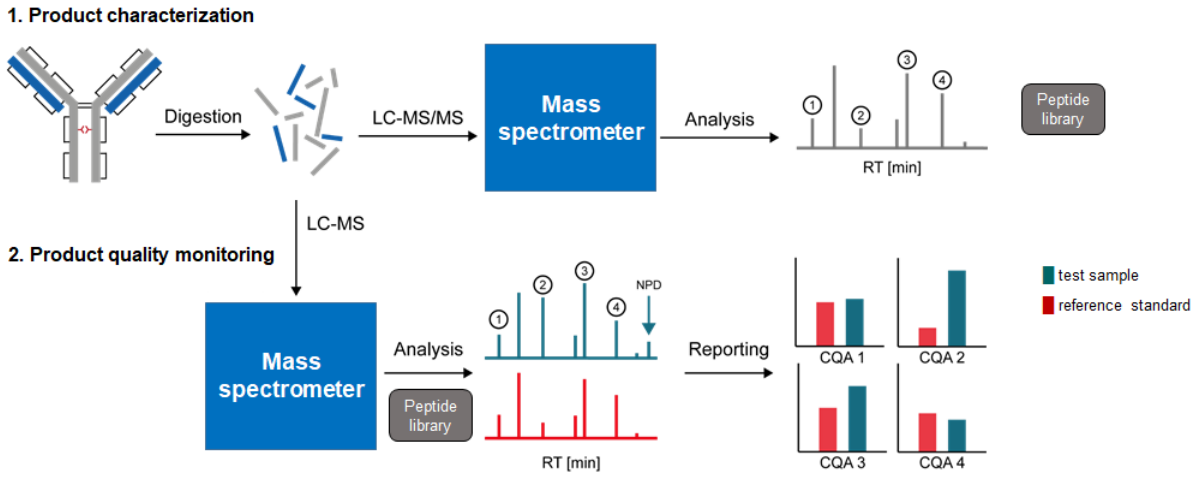


Figure 1: *Schematic overview of the MAM by LC-MS peptide mapping workflow enabling relative CQA quantification and New Peak Detection (NPD) (adapted from Rogers et al., 2018 [2])*

The MAM by liquid chromatography mass spectrometry (LC-MS) peptide mapping workflow that will be the specific basis for discussion in this manuscript is shown in Figure 1. Execution of the MAM by LC-MS method consists of digestion of the biopharmaceutical protein with an appropriate endoprotease followed by LC-MS analysis. Prerequisite for the targeted monitoring of individual PQAs is an initial product characterization by LC-MS/MS to identify product-related variants and to assign them to the individual signals obtained by the LC-MS instrument. Finally, the unmodified peptides and the peptides derived from each product-related variant (modified peptides) are unambiguously described by their chromatographic retention time, mass, and isotopic distribution as detailed in a protein MAM peptide library. The MS signal intensity of the unmodified and modified peptides will be used to calculate the relative abundance of each product-related variant. Peptides derived from product-related variants or process-related impurities (e.g., host cell proteins) that are not contained

in the initial MAM peptide library and that give rise to a well resolved LC-MS signal will be detected by the new peak detection (NPD) feature (see Section 3.4), involving the comparison of signals obtained with the sample to those of a well characterized reference standard [3], [4].

As illustrated in Figure 1, three scenarios should be considered when using MAM as an impurity assay:

1. Monitoring the change in relative abundance of a known species, which is considered a CQA (e.g., CQA2, in Figure 1)
2. Monitoring the change in relative abundance of a known species, which is not considered a CQA (low criticality quality attribute)
3. Detection of an unknown species, which was not observed previously during product characterization studies (NPD), and of undefined criticality.

MAM is frequently applied across the biopharmaceutical industry for product and process characterization purposes, in a non-GMP environment. Although MAM using MS technology is well advanced with instruments and software solutions being available from several vendors, it is not widely used for batch release and stability testing under GMP for QC purposes in part due to regulatory unfamiliarity and limited experience, despite broad interest [5].

The purpose of this manuscript is to promote more frequent regulatory filings of MAM as a QC tool and to improve regulatory understanding. It proposes scenarios for introducing MAM to monitor multiple product quality attributes with one method for QC release and stability testing, thereby replacing several conventional physico-chemical QC methods that indirectly, and often non-specifically, measure the same set of quality attributes. Each scenario is expected to require different elements to support the analytical development and regulatory filings, such as risk assessment supporting the introduction of MAM, potential bridging with conventional methods, method qualification/validation, and specification setting. These elements, as well as handling of new peaks (NPD), are discussed in the following sections.

2 Regulatory pathways to introduce MAM

There are, in principle, no identified regulatory hurdles to file MAM for batch release and stability testing under GMP in a QC environment. Implementation of MAM as a QC tool would facilitate advanced control strategies in line with ICH Q8 [1] and is supported by established and draft ICH

guidelines (e.g., ICH Q2, ICH Q6B, ICH Q14) [6], [7], [8]). Filing of MAM is conceptually not different from other methods and, the benefits of MAM for the control of site-specific CQAs should be seen as an improvement compared to conventional methods (see Sections 3.1 and 3.3).

As for any other analytical test method, regulatory agencies expect a comprehensive understanding of the analytical procedure and adherence to predefined criteria for performance characteristics (ICH Q14 [8]), such as specificity/selectivity, accuracy and precision over the reportable range (ICH Q2 [6]). When using MAM, the applicant is expected to have a thorough understanding of how the performance characteristics of the different methods compare for any given PQA. Regulatory agencies expect comparisons of information obtained by MAM to that obtained using conventional methods, to ensure connection of existing knowledge regarding PQAs and the control strategy that supported earlier phases of clinical development [5].

The conventional methods that could be considered for replacement by MAM, within the scope of this document, are listed in Table 1. Note that methods used to monitor process-related impurities, such as host cell proteins (HCPs), are out of scope of this position paper.

Table 1: *Established conventional methods that could be replaced by MAM by LC-MS peptide mapping*

Quality Attribute	Conventional method
Charge variants	IEX, cIEF, CZE
Fragments	rCE-SDS*
Glycans	2-AB HILIC, HPAEC PAD
Identity	peptide mapping LC-UV, ELISA (in combination with IEX, or cIEF)
Oxidation	RPC, HIC

*SEC or nrCE-SDS could also be used for clip monitoring

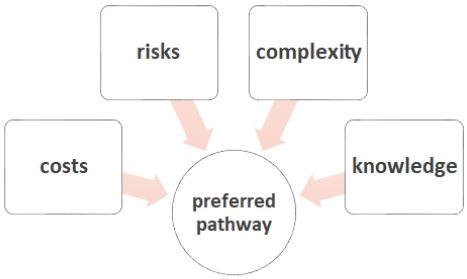
It is not the intention to replace all QC assays with MAM. There are some attributes that cannot be monitored by MAM. High molecular weight species (dimer, oligomers, aggregate), incompletely assembled antibody species, higher order structure, biological activity, and microbiological properties will continue to be monitored using conventional methods such as SEC, non-reducing CE-SDS (nrCE-SDS), biological assays, and microbiological methods, respectively. Alternatively, quality attributes listed in Table 1 can be replaced by MAM if supported by an appropriate risk assessment [5].

For example, replacing a charge-based method (e.g., IEX) by MAM is deemed acceptable for two reasons: 1) MAM is a bottom-up approach that quantifies site-specific modifications, whereas IEX provides an overview of the charge variant profile of the molecule, without identification or localization of individual variants. The charge profile obtained by IEX reflects the overall charge distribution of many individual molecules with different modifications at different sites, meaning that several CQAs can be contained in a IEX single peak. The 'main peak' observed in IEX contains variants with different modifications that likely cancel out the charges relative to its acidic or basic variants. 2) Although IEX is conducted under native conditions and may be able to detect conformational changes, those changes can be better captured by biophysical assays, and their impact will be assessed by the potency assay.

To advance the implementation of MAM as QC tool, the following regulatory scenarios and their supporting elements will be discussed in this manuscript:

- **Scenario 1:** Introduction of MAM during development (post First-In-Human (FIH) clinical studies, but prior to registration) replacing conventional methods.

- **Scenario 2:** Introduction of MAM as a Life Cycle Management (LCM) activity in the commercial phase replacing conventional methods.
- **Scenario 3:** Introduction of MAM prior to FIH studies for release and stability testing instead of conventional methods – Industry-preferred scenario



Introduction of MAM

	Risk assessment	Method bridging	Method validation	Specification setting	NPD*
Scenario 1 – During development – replace conventional methods, where relevant	yes	yes	yes	yes	yes
Scenario 2 – Lifecycle management – replace conventional methods, where relevant	yes	yes	yes	yes	yes
Scenario 3 – Prior to FIH studies – instead of conventional methods, where relevant	yes	no	yes	yes	yes

*NPD : only relevant for non-targeted approach (see section 3.4.1.)

Figure 2: *Scenarios and associated elements for the filing of MAM as a QC tool*

The anticipated challenges and required elements, such as risk assessment, method bridging, method validation, specification setting, and need for the NPD feature in a regulatory filing that includes MAM for QC applications, will depend on the scenario as illustrated in Figure 2. The drivers to select the preferred pathway are cost, complexity of the analytical package, associated risks, and prior knowledge held by the Applicant in using MAM. The Applicant may also choose to implement gradually MAM first as an additional method [14] and later for a replacement of conventional methods as described in scenarios 1 and 2 above. Alternatively, the Applicant may also choose to continue keeping conventional methods for characterization purposes.

For each of the scenarios proposed, it is not expected to replace all conventional methods with MAM as discussed above. Therefore, a conceivable scenario is that the CQA measured by MAM is also indirectly measured by one of the remaining conventional methods (e.g., fragments by SEC or nrCE-SDS). This may in theory result in a different batch disposition status (pass versus fail specifications) depending on the result of each of the two methods. Nevertheless, the associated risk is similar to the

current use of conventional methods, where the same CQA is measured by two orthogonal methods e.g., determination of the relative abundance of the Fab fragment of an antibody by SEC and nrCE-SDS.

In **scenario 1** and **scenario 2**, MAM is intended to supersede certain conventional methods (see Table 1), requiring performance of a risk assessment and a method bridging exercise. The extent of the risk assessment to support the replacement and the required data set to bridge conventional methods and MAM will depend on the stage of product development when the method change is performed. This suggests that for a period of time two sets of methods will coexist. To define appropriate specifications with MAM, it is important to include data obtained from clinical batches to assess maximal clinical exposure of individual CQAs (section 3.1). Furthermore, the replacement of conventional impurity assays by MAM will require the assessment of the performance of the targeted monitoring and NPD workflow to mitigate the risk of not detecting unknown species, which may not be possible until later development when sufficient data are available (section 3.4.2).

Scenario 3 suggests that no conventional methods, which are in scope of MAM (see Table 1) have been applied for the quality control of the molecule. MAM is set up prior to the release and stability testing of clinical batches intended for supply of FIH studies. When considering MAM, this approach is the industry-preferred scenario, as the use of multiple conventional QC methods or the requirement to perform method bridging studies translates into significant resource demand, associated with extended timelines and costs. It is acknowledged that developing MAM for FIH batches potentially requires additional, upfront product characterization to establish the MAM peptide library and to ensure the MAM targets the relevant CQAs. Nevertheless, it is expected that with increasing experience of employing MAM as a platform technology and risk-based development approaches, the additional upfront investment will diminish. Scenario 3 is considered a viable option taking into account prior knowledge regarding common quality attributes for the same structural class of therapeutic proteins (e.g., monoclonal antibodies).

In conclusion, it is up to the Applicant to define which strategy they want to follow based on their risk assessment also considering the prior knowledge of the company on the understanding of the correlation between MAM and conventional methods.

Regional regulatory differences in the acceptance of novel technologies, e.g., methods based on mass spectrometry, could be a challenge. A global regulatory landscape and globally harmonized regulatory opinions would prevent the Applicant from having to maintain two distinct analytical control strategies

in parallel in a QC environment, one based on conventional methods only, the other based on MAM. In case of in-country testing required for certain countries/regions, and no waiver for complex analytical technologies is possible, the state laboratories may require the use of conventional methods in case they are not equipped to conduct MAM by MS methods.

3 Elements supporting introduction of MAM as a QC tool

3.1 Bridging with conventional methods

Introducing MAM prior to FIH studies (scenario 3) would not require any bridging exercise with conventional methods. However, for scenarios 1 and 2, bridging of MAM and conventional methods is expected by regulatory agencies, to demonstrate that MAM is at least equivalent or even superior to the original method for the intended purpose.

The method bridging exercise, if required depending on the scenario chosen, should be supported by a risk assessment [5], and the reportable results of the two methods need to be compared and any observed differences need to be discussed and assessed with regards to their impact on monitoring product quality. The output of MAM is specific to an attribute and its location within the molecule. While conventional methods will provide information at the quality attribute level, they often do not inform at the site level, nor specific chemical nature of the (e.g., deamidation, oxidation) attribute. This can make the bridging of MAM and conventional methods challenging.

Given the differences in the measurement techniques, it is not realistic to expect an exact match between MAM and the conventional method results. Despite this, for many attributes, good to excellent correlation between MAM and conventional method(s) have been described in the literature [9], [10], [11], [12]. MAM has advantages compared to the conventional methods having improved specificity through measurement of a specific quality attribute at a defined location on the polypeptide chain (e.g., deamidation in the heavy chain CDR by MAM compared to 'sum of acidic variants' by IEX). Therefore, in the bridging exercise it may be required to sum the results obtained by MAM at the individual quality attribute level to compare with the reportable result obtained by the conventional methods [11].

The extent of the data package to demonstrate that MAM is at least equivalent to conventional methods will depend on the scope of the method (in-process control (IPC), release and/or stability)

and phase of development. As for any method change, multiple samples/batches covering all relevant sample matrices, including selected force-degraded and stressed samples, should be analyzed to compare the stability-indicating nature of both methods. The new method, in this case MAM, needs to be at least as sensitive as the previous one in detecting relevant degradation pathways of the molecule, changes in attribute level in manufacture, consistency through shelf-life or expiry. In the case of replacement of a conventional method with MAM, clinical (or commercial) batches should be included, where possible, to support the specification setting based on exposure of the CQA to patients. Stability data should be evaluated, to demonstrate similar trends. In most cases, it is not meaningful to compare absolute values, therefore only trends and rate of change of the CQA during long-term and accelerated stability should be compared.

MAM and the conventional method may not generate equivalent data, which is considered acceptable as long as the root cause for the difference is understood. It is important to demonstrate that the data obtained by MAM, although different, are linked to the conventional method data that had supported the clinical phases. Consequently, any relevant difference will be also reflected in the set specification limit for the given attribute monitored by MAM. Successful bridging will ultimately demonstrate the suitability of the MAM approach to control product quality attributes to levels that were previously shown to be safe and efficacious in patients using other conventional methods.

For post-approval changes (scenario 2), the use of the Analytical Target Profile (ATP) defined for each critical quality attribute is encouraged (ICH Q14 [8]). Within the risk assessment, the Applicant should evaluate the potential impact of the change on the method performance characteristics and the link to the CQA as defined in the ATP. Based on the outcome of the risk assessment, experimental bridging studies may be required to demonstrate that the MAM adheres to the performance characteristics and associated acceptance criteria. Finally, the ATP will help in evaluating the impact of the change and selecting the appropriate reporting categories for the replacement of multiple conventional methods by a single MAM.

3.2 Method qualification/validation

To allow MAM to be used in a GMP environment, such as a quality control (QC) laboratory dedicated to the release and stability testing of biopharmaceutical products, the method must adhere to a level of GMP compliance comparable to that expected for other conventional physico-chemical methods, such as liquid chromatography, or methods based on capillary electrophoresis. MAM must be qualified

or validated, as appropriate for the stage of development the MAM is being used to support. In addition, the integrity of the data obtained by MAM (both raw mass spectrometry datafiles and the resulting processed datafiles that are used for reporting eventually) must be ensured.

For application in a QC environment and considering the *multi-attribute* nature of MAM, the method qualification, or validation following the principles of ICH Q2 [6], should cover all critical quality attributes, and may also include NPD. Certain quality attributes may be used as surrogates for others during the MAM method qualification/validation depending on their representativeness, i.e., considering the different behaviors of each peptide (e.g., ionization efficiency) and their relevance for a specific degradation pathway (e.g., Fc methionine oxidation). Grouping of certain attributes could also be considered, for example the sum of all Fc methionine oxidized species (see discussion in section 3.3). Prior knowledge from similar molecules (e.g., IgG1 class antibodies) can be used for MAM method qualification/validation as supported by ICH Q14 [8].

According to ICH Q2 [6], the following parameters should be considered when qualifying/validating MAM as a quantitative assay for impurities: accuracy, precision (repeatability/intermediate precision), specificity, quantitation limit, linearity, and range.

MAM will benefit from being developed according to the enhanced approach principles of ICH Q14 [8], including ATP for each CQA, method risk assessment, identification of method parameter set points and/or ranges, analytical control strategy definition, and routine monitoring. This approach, together with the prior knowledge obtained from similar molecules, will ease the method lifecycle management especially when MAM is introduced as replacement of conventional methods (section 3.1).

The aspects related to validation of NPD are discussed in Section 3.4.

3.3 Specification setting

The same principles apply for setting release and shelf-life specifications with MAM as for any other impurity method for a biopharmaceutical, meaning that acceptance criteria should be defined according to ICH Q6B [7] for relevant quality attributes of the product. Clinical and preclinical experience, the method performance characteristics accuracy and precision (as defined in the ATP), as well as process capability (batch-to-batch variation), and stability profile will be key to inform the CQA specification limit.

The release and shelf-life specifications need to be clinically relevant, meaning that the dose-ranging studies need to be supported by MAM, where possible. If this is not possible (e.g., where MAM is introduced during the development of the product) and given that the values measured by MAM may be different from the values measured by conventional methods (scenarios 1-2, section 2), the bridging study (section 3.1) between MAM and conventional methods will be key to understand any offset and to be able to demonstrate that the MAM values are linked to the conventional method data that were used to support the clinical phases. In the case where MAM is introduced directly for FIH studies (scenario 3, section 2), the specification will be defined without comparison to conventional method data, even though the conventional methods may be used for characterization purposes.

MAM, by definition, measures multiple quality attributes in the same analysis. However, the release and shelf-life specifications should address only those quality attributes determined as critical (CQAs). Prior to pivotal studies the specification could be based on early indicator peptides representative of a certain product quality attribute class. As an example, the peptide containing the methionine most susceptible to oxidation (usually DTLMISR in the Fc part of an IgG [13]) is typically representative for the overall oxidation status of a mAb. Reporting the sum of relative abundance for the same type of modifications with similar criticality (e.g., oxidation in the CDR) could be considered to reduce complexity. If an attribute is defined as a CQA during late-stage development and there is no conventional method available to measure this attribute, the specification could be determined based on the analysis of retention samples with the MAM being properly qualified for the new attribute (section 3.2). Alternatively, the data previously acquired with the MAM from development and stability studies could be analyzed retrospectively to define the specification related to this new CQA. MAM also facilitates the monitoring of other (low criticality) quality attributes, outside of specifications, enabling further knowledge gathering on the molecule for manufacturing consistency within the Pharmaceutical Quality System (PQS).

In line with ICH Q6B [7] requirements, an appropriate subset of methods should be selected and justified for determination of purity. With the implementation of MAM as QC tool some conventional methods (e.g., ‘Purity by SEC’ or ‘Purity by non-reducing CE-SDS’) would remain on the testing panel (section 2) for the assessment of purity in terms of the native, fully assembled, and monomeric antibody. Ideally continuation of determination of ‘Purity by IEX’ or ‘Purity by cIEF’ in terms of the charge variant profile, should not be necessary when MAM is in place, as the main peak observed with those methods is typically not fully representative of the sum of product related substances. The main

peak from IEX reflects a complex combination of attributes on a single molecule. Other product related substances of a mAb (e.g., C-terminal lysine variants) are separated as basic peaks and not included in the calculation of relative purity. The authors conclude that the inherent specificity of MAM is a better indicator of product purity than the IEX main peak.

Implementation of MAM in the context of scenarios 1, 2, and 3 would require performance of the NPD workflow including appropriate specification setting to control unknown impurities (Table 1). Due to the inherent risk of detecting more unknown impurities during early development stages, when product characterization data is still limited, it is proposed to set specifications for NPD in a stage appropriate manner (see section 3.4.3).

3.4 New peak detection (NPD)

3.4.1 Use of NPD

The LC-MS peptide mapping workflow can be used in two ways: either as a targeted method or as a non-targeted method.

- In the targeted approach, only a set of specific CQAs is evaluated by targeting specific m/z values corresponding to the modified and to the non-modified peptides. The MS signal intensities of those peptides are used to calculate the relative abundance of the product quality attribute (e.g., asparagine deamidation, methionine oxidation, glycosylation, etc.). For a targeted approach, NPD is out of scope and the method is commonly also referred to as Multi-attribute monitoring [14].
- In the non-targeted approach, multiple quality attributes are evaluated as well as any new peaks. Criteria regarding the reporting of new peaks should be defined to ensure they are meaningful given the complexity and the sensitivity of the MS technique.

When NPD criteria highlight the presence of a new peak, the underlying peptide needs to be sequenced, e.g., by LC-MS/MS, to elucidate its structure and to enable the subsequent update of the MAM peptide library supporting the later routine monitoring of the newly identified, product-related variant.

During early stages of development, it is very likely that many new peaks will be detected, especially in the course of accelerated or stressed stability studies. These species would be characterized and recorded in the MAM peptide library for the product under development. Therefore, it is expected

that the MAM peptide library will evolve throughout development and ideally will be fully comprehensive in terms of inclusion of all relevant product quality attributes prior to commercialization (post-PPQ batches). To limit the number of new peaks, which are not yet included in the peptide library and detected by MAM during formal stability studies it is recommended to perform forced degradation studies prior to the implementation of MAM in a QC environment.

In those scenarios where a non-targeted MAM approach is implemented post-FIH studies (scenarios 1 and 2), it is strongly advised to retain release and stability samples of clinical batches. In case new impurities are detected by MAM, these retention samples will be required for additional characterization purposes and the targeted MAM analysis to determine the clinical exposure of the impurity. The advantage of employing MAM for release and stability testing prior to FIH studies is that the data are already captured and hence only *in silico* evaluation of the previously recorded MS profiles is required to retrospectively assess the relative abundance of those impurities.

3.4.2 NPD parameters validation

The most important parameters of the NPD workflow that must be defined prior to data processing are the following (see also Figure 3):

- MS intensity threshold
- fold-change threshold
- mass and retention time tolerance windows

Prerequisite for successful performance of NPD using a robust method, is the retention time alignment of sample versus the reference standard and the accurate detection of species with a truly changed abundance, which will require the empirical determination of project-specific NPD processing parameters to minimize false positives and false negatives.

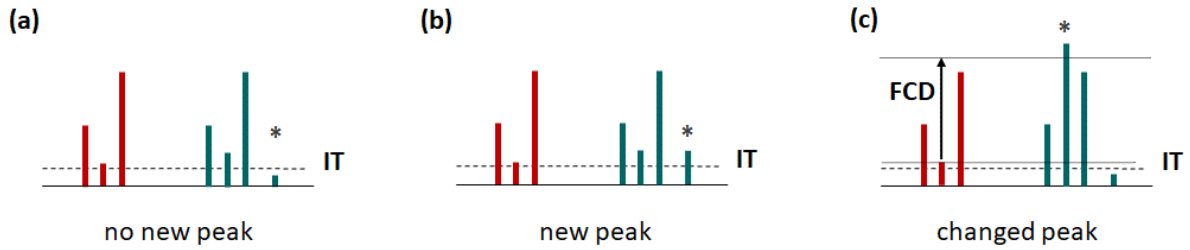


Figure 3: *Illustration of NPD thresholds. MS signals obtained from the reference standard and those of the sample are shown in red (—) and green (—) respectively. An additional signal (*) observed in the sample compared to the reference standard will only be reported as a new peak by the MS software if its MS intensity will exceed the pre-defined MS intensity threshold (IT) (see insets (a) and (b)). If the MS signal intensity of a peak observed in the sample exceeds the MS signal intensity of the corresponding peak in the reference standard by a predefined factor, i.e., the fold-change detection (FCD) threshold, this peak would be reported by the MS software as a changed peak (see inset (c)).*

NPD parameter validation will be performed when the MAM peptide library is considered comprehensive, typically, at the time of process performance qualification (PPQ). The empirical determination of NPD thresholds can be based on spiking varying amounts of synthetic peptides, or peptides resulting from a digest with an enzyme that has different specificity from the enzyme routinely used for MAM (e.g., chymotryptic peptides in a tryptic digest). While synthetic peptides could be used as surrogate, e.g., deamidated or oxidized species, the latter could be used to mimic backbone clipping. While common mass and retention time tolerance windows are applied for multiple molecules throughout the industry [3], the MS intensity threshold (IT) and fold-change detection (FCD) threshold may be different from molecule to molecule and would be qualified once sufficient experience has been gained with the application of MAM for the specific product.

3.4.3 NPD and Specification

As outlined in section 3.3, it is considered advisable to set specifications for NPD in a stage appropriate manner to mitigate the risk of inappropriate batch rejection and delayed drug supply to patients. In the context of the specification setting, a MS signal should be only attributed to a “new peak”, if it exceeds any of the NPD thresholds (see Figure 3) and is not already included in the MAM peptide

library. Therefore, during product development, with increasing product quality attribute coverage by the MAM peptide library, the potential risk associated with NPD regarding batch false rejection will decrease.

For the QC release of clinical batches, a two-tiered NPD specification approach may potentially be used for batch disposition, based on the definition of an alert and action limit. These definitions would be company-specific's and the following are provided as examples only:

- Exceeding the **alert limit** has no impact on batch disposition but will trigger peak characterization.
- Exceeding the **action limit** will put the batch on hold and will trigger an investigation, including peak characterization and the assessment of a potential impact of that species on the safety and efficacy of the product.

The alert and action limits may be compared to the MS signal intensity of the newly detected peak and should be set to balance the risk of not detecting unknowns with a potential impact on the safety and efficacy of the product and batch rejection based on a false positive result. The peak characterization information could be used to update the MAM peptide library as shown in Figure 4. This approach would be no different to setting a relatively wide generic specification limit at early development stages for a conventional method, e.g., 'Sum of acidic variants by IEX", with the company's internal requirements to investigate should a previously unknown peak be detected within the chromatogram.

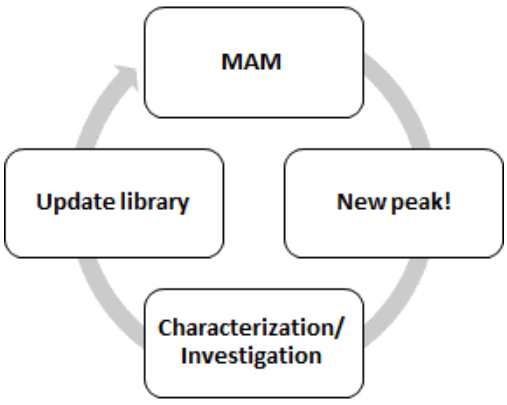


Figure 4: *Stage-appropriate evolution of MAM peptide library following NPD*

For post-PPQ and commercial batches, the presence of a new peak may suggest an issue with the batch produced, hence an investigation would likely be triggered prior to releasing the batch. The NPD parameters would have been validated at this stage (section 3.4.2) and the MAM peptide library considered comprehensive in terms of inclusion of all relevant product quality attributes. Therefore, if the new peak is not referenced in the library, characterization should be required to understand whether it is a product-related substance/impurity or a process-related impurity such as a co-purified HCP. The outcome of the characterization should inform the criticality assessment of this species in terms of its impact on the safety and efficacy of the product, which in turn will impact the batch disposition decision and the need for inclusion in the stability program if the species is stability-indicating. Any other peak, which exceeds the NPD thresholds but is included in the MAM peptide library and is not considered a CQA, may be trended to support the company internal process consistency monitoring program.

4 Conclusion

In conclusion, the authors have not identified any regulatory impediment to introducing MAM into the overall control strategy for a biopharmaceutical, whereas the outcome of using MAM has advantages compared to conventional analytical methods. Thanks to the method's unique ability to assess individual site-specific critical quality attributes (CQAs), MAM by MS approaches allow a better-informed process parameter risk ranking and attribute range setting and facilitates the rapid development of CQA-focused control strategies that will ultimately ensure more meaningful quality control of pharmaceutical products and potentially shortened development timelines for earlier patient access.

Earlier introduction of MAM reduces burden of historical method/data bridging. It is acknowledged that introducing MAM prior to FIH studies translates into additional, initial effort but the knowledge gained would aid subsequent stages of development. In the longer term, with increasing experience of employing MAM as a platform technology, it is expected that the work required to introduce MAM would be not different to the development of a set of corresponding conventional analytical methods.

MAM should be treated like any other analytical method following a program of development, optimization, and qualification/validation with a level of detail proportionate to the stage of product development. Similarly, MAM specifications should be treated like 'conventional' specifications, i.e.,

they should be justified by batch and stability data, product and process understanding, method performance, and by clinical exposure.

Additionally, being a highly specific method to monitor CQAs, MAM allows the gathering of in-depth knowledge on known product quality attributes, from a single analysis, as well as the detection of unknowns by NPD. It is also possible to re-interpret the MAM data retrospectively upon accumulation of knowledge about a product's (critical) quality attributes, without necessarily having to perform new analyses on retained samples.

The introduction of MAM in a regulatory filing for QC applications may require significant initial resource by the Applicant but it offers advantages as described above. It is for the individual companies to decide what approach they will follow based on product risk assessment and prior knowledge. In this context the Applicant should be encouraged to engage with different regulatory agencies to seek for advice and for feedback on any filing using MAM as a QC method.

5 References

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6 Glossary

ADC	Antibody Drug Conjugate
ATP	Analytical Target Profile
CDR	Complementary-Determining Region
cIEF	Capillary IsoElectric Focusing
CQA	Critical Quality Attribute
CZE	Capillary Zone Electrophoresis
DS	Drug Substance
ELISA	Enzyme-Linked Immunosorbent Assay
FCD	Fold Change Detection
FIH	First In Human
HCP	Host Cell Protein
HIC	Hydrophobic Interaction Chromatography
2AB-HILIC	2-aminobenzamide – Hydrophilic Interaction Liquid Chromatography
HPAEC PAD	High Performance Anion-Exchange Chromatography – Pulsed Amperometric Detection
ICH	International Council for Harmonization
IEX	Ion Exchange
IPC	In-Process Control
IT	Intensity Threshold
LC-UV	Liquid Chromatography – Ultra-violet
LOD	Limit of Detection
LOQ	Limit of Quantification

MAM	Multi-Attribute Method
MS	Mass Spectrometry
NPD	New Peak Detection
nrCE-SDS	Non-reducing Capillary Electrophoresis – Sodium Dodecyl Sulfate
PQA	Product Quality Attribute
PQS	Pharmaceutical Quality System
PTM	Post Translational Modification
rCE-SDS	Reducing Capillary Electrophoresis – Sodium Dodecyl Sulphate
RPC	Reverse Phase Chromatography
SEC	Size Exclusion Chromatography

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