IPQ INTERNATIONAL PHARMACEUTICAL QUALITY
Inside the Global Regulatory Dialogue

Amgen "Analytics of the Future" Initiative Develops MS-based "Multi-Attribute Methodology" to Streamline Biotech Development and QC Testing

Amgen is among the major biotech companies working hard on empowering, while at the same time streamlining, their analytical toolbox at the development and quality control testing levels as the next frontier of quality by design.

How to realize "the promise of analytics of the future" using what Amgen is calling "multi-attribute methodology" (MAM) was the theme of a presentation by the biotech company's Contract and Product Quality VP, Anthony Mire-Sluis, during a session focused on QbD implementation challenges at CASSS' CMC Strategy Forum Europe held in Sorrento, Italy in May 2014. Prior to joining Amgen in 2004, Mire-Sluis played a key role in biotech CMC policy setting at FDA.

At the session, he revealed: ● the purpose and rationale of Amgen's MAM program ● how the vision was pursued using mass spectrometry (MS) methodology ● examples of its power in assessing product quality attributes, and ● the regulatory implications in moving to its use in specification-setting and routine QC testing.

> **The presentation shed light on the issues that come to the fore as the rapidly advancing analytical technology pushes up against existing biotech regulatory policies and on the adjustments that need to be made to accommodate these advancements. As such, it speaks to the intent of the CASSS CMC strategy forums as a vehicle to bring industry and agency experts together to explore biotech developments and the regulatory concerns they engender.**

Also presenting at the session were Sweden Medical Products Agency (MPA) biotech product assessor Mats Welin, who offered "a regulator's view" on "how to assure quality and consistency in an evolving QbD scenario," and MedImmune UK's Derek Murphy, who addressed the impact of QbD on QA and the Qualified Person (QP).

A significant discussion period followed the presentations that focused heavily on Amgen's single multi-attribute method approach and the regulatory issues it raises.

Sorrento Forum Explores Regulatory Impact of Cutting-Edge Biotech

The 2014 CMC Strategy Forum Europe began with a plenary session at which key regulators from Europe, the US and Japan provided updates on current regulatory initiatives and trends in the biopharmaceutical arena.

A series of four workshops followed on emerging aspects of CMC technology and regulation. Along with the session on the advancing QbD paradigm at which Mire-Sluis spoke, the other workshops focused on: ● comparability and biosimilarity ● the evolving regulatory approach to biotech drug substance process validation, and ● the challenges in managing global supply chains and registrations.

Closing the forum was a plenary session on innovations and initiatives to accelerate product development and clinical evaluation and their regulatory implications. The session explored some of the cutting-edge approaches that regulators will be reviewing, such as deferred cloning, platform manufacturing processes, and new expression technologies.

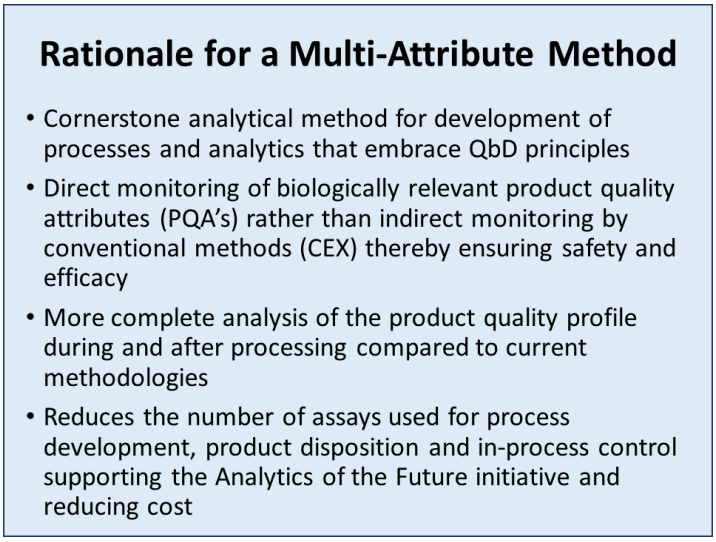
As has been the case for previous European strategy forums, the Sorrento forum was preceded by a "satellite session" sponsored by European Biopharmaceutical Enterprises (EBE).

The session was centered around the work EBE has been doing to better understand the role of, and develop best practices for, forced degradation studies and visible particulate analysis for therapeutic proteins.

One Method, More Power

The rationale behind Amgen's MAM development program, Mire-Sluis explained in his presentation at the CASSS forum, was "to have a single method with the power to be able to be literally the cornerstone for our process development and our product characterization."

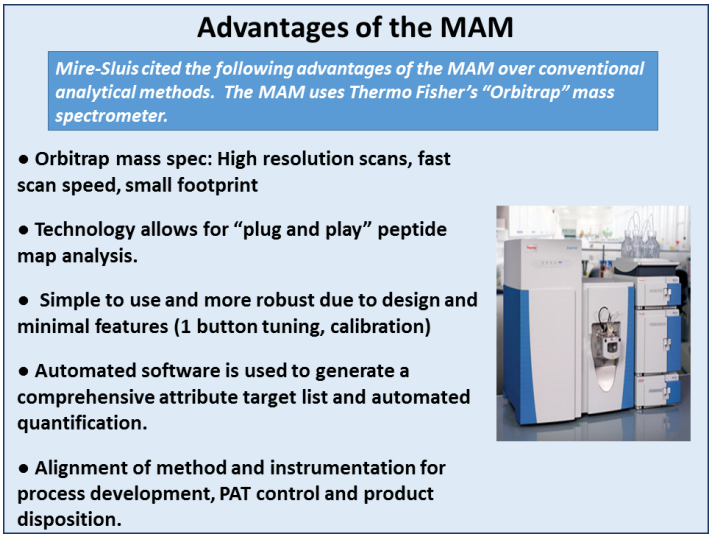
Given the limitations in the current array of general methods in assessing product attributes, Amgen targeted a single method that could measure directly, for example, oxidized, deamidated, and glycosylated protein forms. The company also wanted a method that could be used on line – providing the ability to monitor what is happening to the product as it is being produced.



The overall goal of Amgen's "analytics of the future program," Mire-Sluis explained, is to "really move through QbD to more modern methodologies – trying to get rid of gels and other methods that aren't particularly useful," and replace them with methodology "where you are actually measuring the attributes that you think are important." QbD efforts are focusing on criticality of attribute assessments and need to be supported by a method that can "actually pick up those particular attributes, rather than ones that are more generalistic."

Highlighting the gaps in the ability of the standard approaches to assess various quality attributes, Mire-Sluis explained that the MS methodology Amgen has developed "not only does it all in one go, but actually covers a whole host of attributes that you can't actually routinely detect" using them.

During process development, he stressed, "to be able to get that information in real time with that amount of power is very valuable for understanding your process, which is, as you know, a big part of quality-by-design – true process understanding and building a robust process."



Along with more complete analysis of the product quality profile and critical quality attributes (CQAs) during and after processing, the multi-attribute method allows for reducing the number of assays used for process development, product disposition, and in-process control.

The Amgen expert went on to explain the challenges his firm has been addressing in: ● gaining the "extensive knowledge" needed of the method's capability in view of its high sensitivity ● validating it ● correlating the MAM with current release methods, and ● incorporating it into regulatory filings.

At the Regulatory Interface

Amgen's initial regulatory strategy is to file the MAM as a characterization method – using it alongside rather than inside regulatory specification setting.

For less sophisticated regulators, Mire-Sluis commented, it is challenging enough to "explain the normal methods that we use. So we are going to start off with having it as a characterization method to get everybody feeling more comfortable with it. Then probably by the time it gets to licensure, we would replace the old methods with this single method moving forward."

Amgen does believe that it has put in place the fundamentals to move to the single multi-attribute method for specification use.

The automated MS quantification technology is "very flexible" and usable in a QC environment, Mire-Sluis stressed. "It is amazing the amount of information you can get out of this machine that you can use for process development, for characterizing your product, as well as using it for lot release and stability."

> **The MAM does more targeted assessment of CQAs, allows for better product control for patients, and reduces the cost of quality. Another benefit is its universality, the Amgen official pointed out.**

"So once jurisdictions around the world get used to this type of methodology, import testing becomes much easier when you are working with regulatory authorities that like to retest your material during development. It is a single method, rather than transferring eight or nine methods around the world to multiple jurisdictions and paying for it and sending it to everybody. Once these regulatory authorities have these mass-specs, it should be easier to transfer tests around the world."

MAM Meshes with Understanding Clinical Import

During the discussion period that followed in the session, Mire-Sluis commented on how the MAM interfaces with the focus Amgen has had on understanding the clinical import of variations in the test results given the increasing sensitivity the methodology offers.

As Amgen has been doing with the traditional methods, the read outs are assessed in view of Amgen's learnings about what happens to the product in the patient based on serum analysis ***(IPQ January 16, 2013)***.

When you take product molecules back out of patient serum to see what is actually happening *in vivo*, he said, "you find that lots of things you have been worried about, you don't need to be worried about. I gave this example before: We were very worried about deamidation until we took it back out of the patient and found that, within half an hour, 100% of the molecules were already deamidated. So who actually cares about controlling it to .1% or .5% or whatever it was in the example?"

> **The MS technology can be applied in doing the antibody analysis on patients to expand this realm of understanding.**

The finding, for instance that "deamidation is spread all over the place," means that "we are not really worried or focused on one particular area." While the criticality of variations such as "an oxidation here, a deamidation there" may not be predictable, "at least it gives us the ability to have those discussions. Maybe we will learn something. Maybe we will finally see what immunogenicity is really about. Maybe we can learn something about the function and structure of molecules that we have never seen before."

Amgen, he added, truly believes, for example, "that if we can do comparisons during development, side-by-side with CEX [cation exchange chromatography], you wouldn't have to run CEX anymore." If you know you are controlling the attributes that matter and can correlate the methods, he said, "I can see that those old methods will eventually go." Like the process of replacing gels over the years with more sophisticated methods, "I just see this as a natural progression. But we have to prove to the agency that truly we are picking up what the old methods used to."

Pairing species that we know we can isolate from orthogonal methods and comparing them to what we are finding in this MS method is "going to be a learning curve," Mire-Sluis commented when asked for further input on this comparison process.

As with any mass analysis, the significance of the various sequences that are found need to be understood in a holistic manner. However, Mire-Sluis affirmed, "it is amazing what mass-spec can do. I have seen mass-specs now that could replace size exclusions or could do aggregates. This is one of those fields where you just watch the technology that is out there. I can't predict whether this is going to be the only test we ever use, but you know what, science is an amazing thing. In ten years times, we may find a single assay that does the whole lot."

> **Another issue that was raised during the discussion was Amgen's reliance on sourcing from a single MS and software provider.**

Mire-Sluis acknowledged the danger in a single source supplier disappearing. However, the awareness of this type of MS technology and supporting software is expanding, and similarly to the progression in CE, over the next few years there will be other players "getting onto the same bandwagon," he affirmed.

While more sophisticated than the gel technology, as regulators get acclimated, "hopefully more vendors will start looking at this stuff, and we will get more software people involved."

How Should MAM be Sold to Regulators?

Biogen Idec Biopharmaceutical Development VP Rohin Mhatre suggested that it would be to the advantage of the regulatory community for Amgen to position the MAM program as an extension of, rather than a radical shift in, the analytical arena to get more ready buy-in from regulators.

"It is harnessing the power of mass-spec" in the decades-old pursuit of peptide mapping, and is a "very reasonable approach" that "shouldn't be a big hurdle" if sold in that light, Mhatre maintained.

> **Lilly Regulatory Advisor John Dougherty, the session moderator, asked Mire-Sluis to comment further on use of the methodology in the PAT context – "the whole feedback loop of the equation." He cited Mire-Sluis' example of monitoring a cell culture and finding that oxidation was occurring more rapidly than normal. "Would you understand what levers to pull in the actual bioreactor to kind of slow that down then?"**

The Amgen official explained that "that is a very interesting discussion that we have been having in the company. We call it product attribute control." There are people in development, he noted, that feel that as long as the product is what it is supposed to be, "what you do with the process doesn't really matter." He added that the separation-free mass-spec methodology has the ability to characterize the process going on in the bioreactor.

Referencing Mhatre's comment, he stressed that the new part of Amgen's effort is "trying to create a method that can actually be run routinely in the [QC] lab. The software wasn't previously available."

A question was raised on relating the deamidation, amino acid and glycation findings from the standard peptide mapping with the total overall variance.

"That is where all the work comes in" that he discussed in his presentation, Mire-Sluis responded. "It is all about proving your orthogonal methods are showing you stuff the new method is showing."

Noting the power of the Amgen MS methodology in understanding the molecule, he queried whether instead of "adding it up" to find that your product is 10% deamidated, one instead says "'I don't care about the deamidation that is in the FC portion as it has no FC function….' Those are interesting discussions that we are going to have to think about once we move this forward. Do you even take a site of oxidation or deamidation as the thing you want to have on the spec?"

Upfront Work Speeds Up Process Later

Another participant noted that he was "really impressed" by the Amgen methodology as "bringing a lot of new things" to the table.

He pointed out that during development, the process entails "a fair amount of adapting into a certain molecule, programming to a new peptide, and setting new parameters etc. So I assume that you have a significant investment at one point in your development – which is probably at a later point when you are quite confident that this will play out" and be useful for control, manufacturing, and changes.

Mire-Sluis responded that there are definite advantages to doing this type of analysis upfront. Another option is to wait until you know the product works and then "run like crazy" at that point.

While speed to first-in-human studies is important, if a company has not done a good portion of the product and process characterization, the time from first in humans to marketing application can stretch from five to seven years "because you have to catch up."

Companies are going to have to make the decision whether to spend less up front, or do more. And if the product "dies, it dies, but if it works we can get it to patients much quicker." At Amgen, the argument is being made to upper management to buy in on the up-front investment strategy.

Early Discussions with Regulators Recommended

Sweden's Welin commented that how the specifications would be set in the MAM context would need "some discussion."

In general, he said, the regulator would need to be convinced that a new approach is equal or better, and it is up to the sponsor to do that. The challenge for both the assessors and the inspectors is how to learn more about these methods, he maintained.

FDA Center for Drug Evaluation and Research (CDER) biotech reviewer Sarah Kennett, who presented FDA's perspective on recent regulatory trends for biopharmaceuticals in the opening

session of the CMC strategy forum, suggested that these discussions could be fostered by sponsors coming in for a Type C meeting before submitting a marketing application or post-approval supplement.

"I think it is going to take a lot of thought for the agencies to get their heads wrapped around this," she said, suggesting that it would help to have information and discussions up front.

Roche's Kowid Ho, who joined the company in late 2013 after serving as a key CMC policy official for the French agency AFFSAPS and on EMA's Biologics Working Party (BWP), commented that getting the new methodology registered would "not be so difficult" over time.

"There will certainly be some period where the industry may actually have to test the more traditional and new methods until the agencies get sufficiently satisfied with the methods before we drop the more traditional ones," as has been done "for many other new techniques." Such an approach, Ho said, "is not new."

Welin pointed out that the issues involved draw in the larger relationship between the CMC and GMP components of the regulatory equation. Under discussion in Europe, he pointed out, is how the quality system may be used to help assessors become more comfortable in bringing the new technologies on board and setting wider specifications rather than narrowing them based on process capabilities.

The challenge is working with inspectors "to do this in a proper way, using…not only what is in the file, but also what is in the GMP for us to be reassured that these things are under control and there for release."

AMGEN'S TONY MIRE-SLUIS ON HIS FIRM'S MS-BASED MULTI-ATTRIBUTE METHODOLOGY PROGRAM

In a session focused on QbD advancement at the CASSS Europe CMC Strategy Forum in May 2014, Amgen's Tony Mire-Sluis discussed how to realize "the promise of analytics of the future" using what Amgen is calling "multi-attribute methodology." In the presentation, he covered: • the purpose and rationale of Amgen's MAM program • how the vision was pursued using MS-based methodology • examples of its power in assessing product quality attributes, and • the regulatory implications in moving to its use in specification-setting and routine QC testing.

So in this session…we are going to be talking about QbD and some of the potential challenges of implementing it in what I call the real world.

One of the things I wanted to talk about was around the analytics portion of QbD and how in Amgen we are trying to advance our analytics – some of the work we have been doing, but also the potential challenges as we move forward with this once we start implementing it in our filings.

So what I am going to be talking about is the multi-attribute method [MAM] that we have been creating at Amgen:

- What its purpose is and what we believe is the rationale behind it.

- How we actually developed it, because I can assure you it wasn't easy. It is a mass spec method. Mass spectrometry is something that as we wanted to move from the more process development side into the QC labs is not easy to do, because it is complex technology.

- Some examples of what this method can actually do for us from a measuring of product attribute perspective.

- And then some of the risks working with an MAM method, working with regulators and putting this into filings, as well as, as I said, making it a routine part of what we do in the QC world.

The Purpose/Rationale

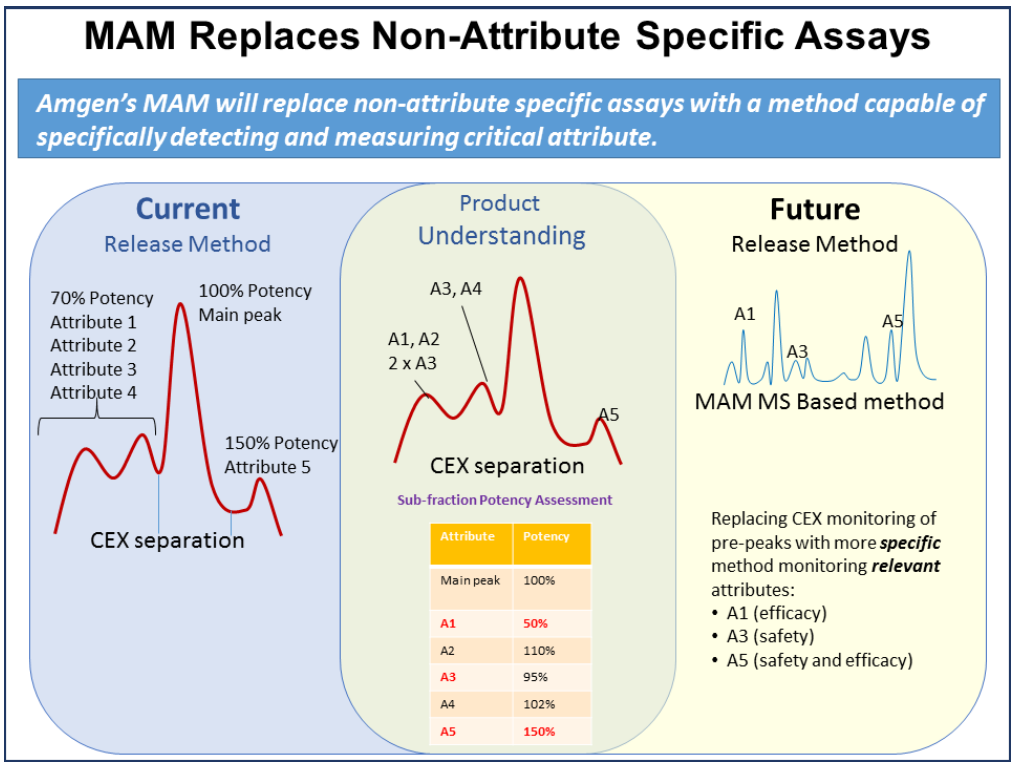
So what was our rationale? Well, we wanted to have a single method with the power to be able to be literally the cornerstone for our process development and our product characterization.

At the moment, most of you are probably aware that most of the methods we use for lot release [are] general methods from an attribute perspective. For instance, CEX gives you acidic peaks, basic peaks, and this thing in the middle. But there are many attributes within those peaks, and it doesn't tell you what they are. It just says you have a certain amount of basic, a certain amount of acidic, and something in the middle.

What we wanted to do was find out if there is a method out there that could actually measure directly oxidized forms of the protein, deamidated forms of your protein, different glycoforms – that sort of thing. And this is where we were moving to with these types of methods.

We also wanted a method that you could actually use online. So you could monitor what is actually happening to your product as it is being produced, following through the cell culture from day one, to day five, to day ten. Then looking during purification to see what is actually happening to your product quality attributes, rather than do as we do now and take those peak profiles of things like CEX, take those individual peaks, and push them through another round of analysis to identify what is within them. That is very time consuming and really isn't something that we could adopt routinely, whereas this [MAM] method hopefully will be something that you can do during development.

Of course, we have at Amgen something that is called the 'analytics of the future' program. [The goal] is to really move through QbD to more modern methodologies – trying to get rid of gels and other methods that aren't particularly useful, to ones where you are actually measuring the attributes that you think are important. A lot of us in QbD are doing criticality of attribute assessments. Well don't you want to have a method that could actually pick up those particular attributes, rather than ones that are more generalistic?



So that was the purpose of us looking for the sort of method that we can use to essentially achieve that type of ideal. As I mentioned, currently the sort of CEX separation you have are the various peaks, and within peak one you are going to have a certain number of attributes, within post peaks you are going to have another attribute, and there is something going on in the main peak.

Well, what you can do, which is what we have been doing in the past, is taking those peaks and essentially measuring, for instance, the potency of them – Is it important? Is it not important? What is the portion of biological activity? – but also what actually are all these attributes?

We want to move away from that. Rather than saying, 'I have got 50 % basic peaks' – you could, in theory, within that 50 % have huge variations in levels of oxidation, of deamidation, or whatever – actually going to a multi-attribute based method that says, 'I have this amount of attribute one, this amount of attribute three, this amount of attribute five.' You can link those to the potency estimates and say which one is important, which one is not important, which is critical or less critical.

I think it is really moving us forward to measuring things that are actually important for safety and efficacy and for the patients, rather than relying on these types of things, which, to be honest, are a bit more bland. You don't exactly know what is going on beneath those peaks – [what] caused a peak to go up. You don't know until you split it up and do a whole lot of other tests.

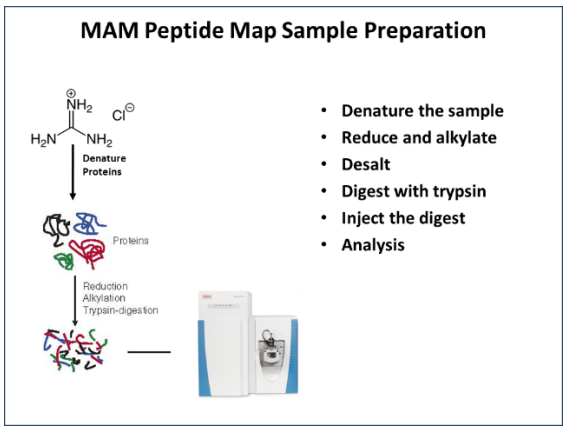
The Development

So what have we developed? We have worked with the manufacturers of a mass-spectrometry machine – you will see here we have an Orbitrap mass spectrometer – as well as a developing the software. This is very

important. There was a great collaboration between us, industry, and the vendors of this equipment without which we could never have gotten this achieved.

It is very high resolution, has a fast scan speed, and a small footprint. You don't want a mass-spec that fits into the size of a rugby field to get that into the QC lab – or actually where we want to put it is on the floor in the manufacturing plant. Our new plant in Singapore, which is built around manufacturing using future concepts, is actually going to have a lot of testing on the floor directly attached to the manufacturing process.

It allows for plug and play peptide map analysis. It is very simple to use…. Mass-spec used to be in the world of the technical experts. They were the only people who knew how to use it. We needed to create something that could be done in a QC lab or on the manufacturing floor. So it is done in a way that you literally push a button and it auto-calibrates and will tell you whether it is okay to put the sample in. And an analyst, be it somebody who is on the floor or someone in QC, just puts the sample in. It has automated software that is used to tell you what attribute you should be looking for.



Obviously, our process development people who are analytical experts are the ones who will set up that program. But once it is ready, the answers that come out the other end are, 'you have 10% of oxidized material, you have 15% of deamidated – something that is really easy for the QC analyst or someone working on the floor to be able to push that data into your laboratory information system and come out with specs or whatever you want to use it for in the future.

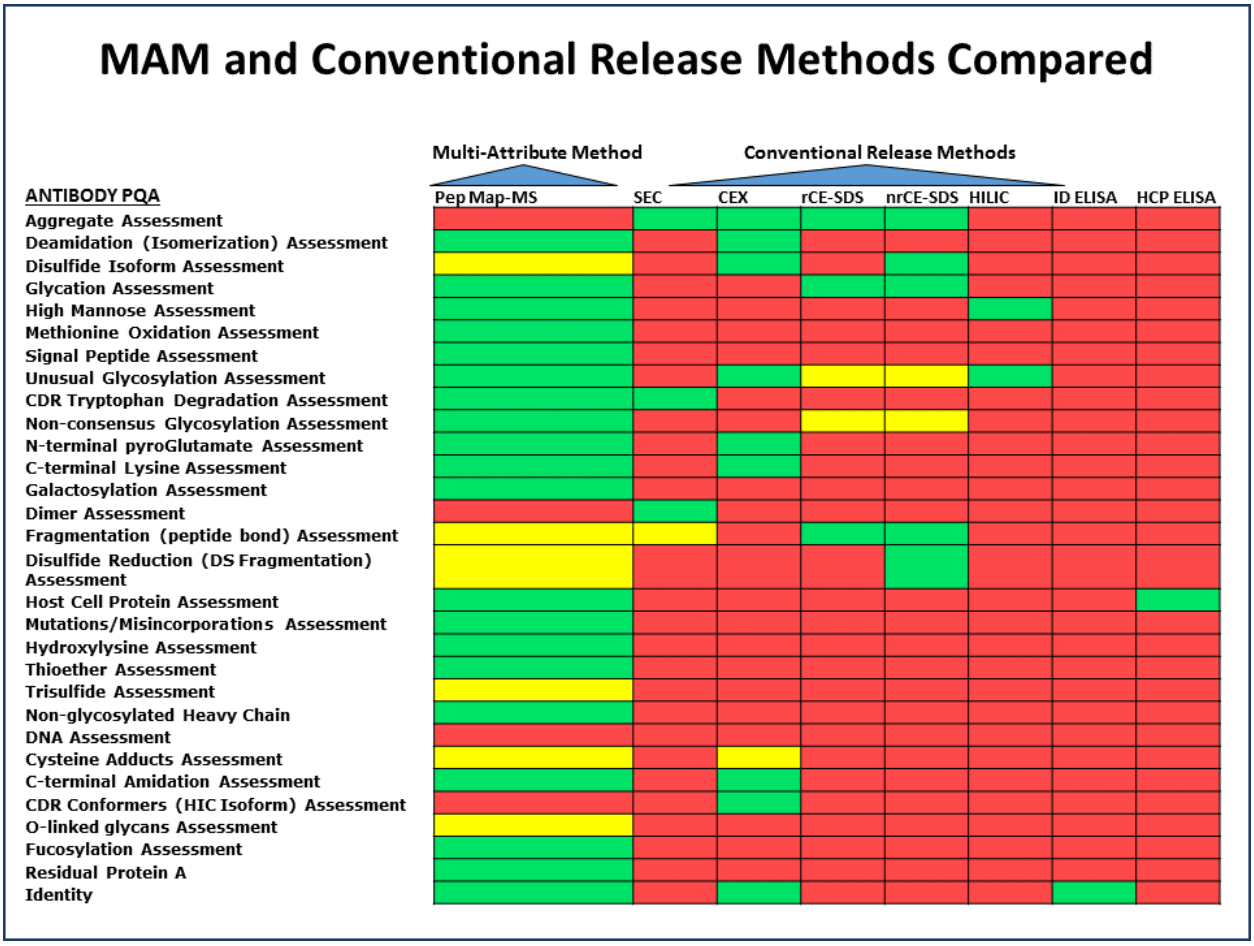
What we want to do is make sure that the method and instrumentation is useful across process development, PAT. You want to use this to make decisions on the floor at the time of manufacturing – looking at the cell culture process, looking at how purification is going, as well as eventually product disposition. So you really need to have the ability to cope with all those different scenarios.

Most of you are probably aware how you do **peptide map analysis**. Essentially, you take your proteins, you denature them, you reduce them, alkylate them in digestion, and then it goes into the machine. So pretty simple, really. Well it sounds simple. Saying that, you have got to make sure that those steps are well controlled enough so that you don't get artifacts through arbitrary trypsin digestion or over-digestion or under-digestion.

To be honest, that is probably where most of the work actually occurred – trying to make sure that we had very robust handling processes – because that is the piece that the people in the QC labs are going to have to be doing on a routine basis. The machinery itself, ironically, once we had been working with the vendors, was kind of the easy piece to sort out.

What the MAM Can Do

We ended up with a process that seems to work reasonably well. The advantage of this – if you look down the product quality attributes you can have for a monoclonal down the side, and there is a whole ton of them – is that this single method can give you information about essentially 95% of all the attributes you want to look at for a monoclonal antibody…. Whereas for the conventional release method, you always need one or the other to tell you what is going on. In fact, you need the whole load of them to cover the whole aspect.



And, in fact, if you look there are whole gaps even in the current methodology that we have around various quality attributes. So this method not only does it all in one go, but it actually covers a whole host of attributes that you can't actually routinely detect on our routine specifications that we currently have in place. So this is a very useful and hopefully more valuable way of looking at products moving forward.

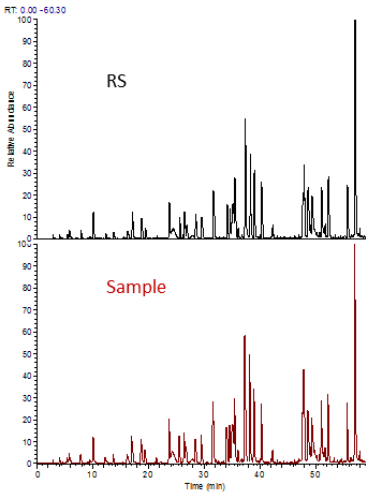
What you would normally get out of CEX [is] a number that has a certain percent acidic, a percent main, and a percent basic. Well, what we can now get is all these different attributes, and more of them, coming out of that single assay. It will tell you what is in this peak, what is in the main peak, what is in the basic peaks, the oxidation types, and where they are. It is not just how much oxidation you have, but where it is in your protein that the actual oxidation occurs. So you can imagine, this is a ton of really useful information.

Not just for specifications, and we will get into that later, because honestly do you really need all this information to release a lot? But you can imagine during process development – developing your fermentation conditions and all that stuff – to be able to get that information in real time with that amount of power is very valuable for understanding your process, which is, as you know, a big part of quality-by-design – true process understanding and building a robust process. This is really helpful for that.

It could also serve as an ID assay. You could imagine the readouts of this machinery are very complex. It truly tells you whether it is the monoclonal that you want. Obviously, there are similarities between monoclonals. That has always caused a bit of annoyance on how you do an ID test. Is it a combination of the potency assay or mass spec or whatever else?

MAM Serving as ID Assay

When MAM is used as an ID assay, the readout of product-specific complementarity-determining region (CDR) peptides ensures specificity.



Parameters	
System Suitability	ΔRT of reference peaks
	Total peak area of reference peaks from RS runs
	%RRA (ratio of relative peak area) of reference peaks
	Mass accuracy < 5 ppm
	S/N for reference peptide ≥ value (TBD)
	The levels of PQAs (2-3) monitored from product specific reference standard within historical range
Sample Acceptance Criteria	Total peak area of reference peaks from sample runs
Sample Pass/Fail Criteria	ΔRT of sample reference peaks
	%RRA of sample reference peaks
	Mass accuracy < 5 ppm
	No new peak above IL limit

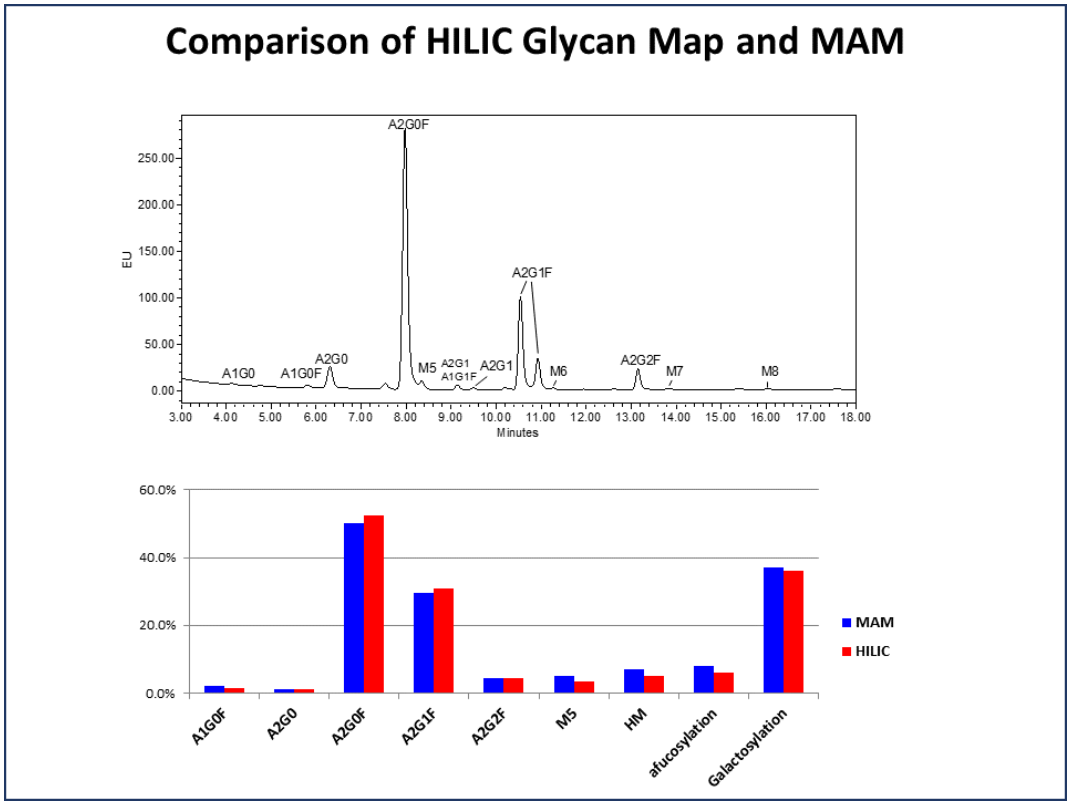
Here you get a one-stop shop, because it is so sensitive. You can truly tell the ID fingerprint. However, you still have to be careful, because there is variability from run to run and variability from lot to lot.

It took us a while to make sure that we got the system suitability right – how much noise can you have in the system to allow you to truly ID the product? Because you can imagine a slight variability in some of these small peaks, and the machine kicks out [that] it is not the same. How similar does it have to be? We did a

reasonable chunk of work to make sure that our acceptance criteria are going to be appropriate so that we truly were looking at identification.

We, as you do, when you are creating a new method, compare it to pre-existing methods. Here we have comparing the glycan map with a method a lot of people use, the HILIC method, and we get really nice agreement across the board with all the different glycoforms that we were looking at.

So you can imagine whenever you are going to file something new with the agency, you always have to compare to existing orthogonal methods to show that what you are really picking up is actually what people are used to seeing. That is part of the development that we do, and will be included in the packages that we provide to an agency.



Assessing Product Quality Attributes

Where does it really start helping? Well, tomorrow, we are going to be talking about all different sorts of control strategies, and that is really where this really starts helping.

Most people do an assessment to look for the criticality of the product quality attributes. Well what actually are your product quality attributes? You can see that with this method. You first of all can detect with a single method a whole ton of your product quality attributes all in one go.

So when you are trying to do those experiments – to say, ‘okay, here is my list of quality attributes. What sort of severity do they have?' – you kind of do them off line, and then go, 'how does my process influence those

quality attributes?' You can actually do that all in one go with this method, rather than every single time going, 'okay, oxidation – I now have to take out that basic peak and find out what the level of oxidized material is in there.' You can do this all in one go as you are doing your process characterization.

So you know that when you do these risk assessments it is always: ● the severity of the potential of your critical quality attribute ● the process capability of either controlling that attribute or causing it to go up or down, and lastly ● the detection. And that is really where this multi-attribute method helps.

Because if you look at the standard way that you would look at methionine oxidation, just as an example, on our calculations, because you can't directly detect oxidation through current specification testing, it becomes red. Because you can't say, 'oh, I know the levels of oxidation at lot release.' You don't. You just have a basic peak, in which could be deamidation, oxidation, or god knows what else. You don't actually have the level of oxidation.

But if you can monitor it over time, and you can actually see the levels of oxidation, that gives you an understanding of how you are controlling it during your process. Even better, if you put it on the specification, you know exactly what the patient is getting from an oxidation perspective. We don't have that with traditional specification lot release. You get an idea of general level based within those peaks that you have, but you don't have an actual number.

Here you can actually say, 'I have been giving, during clinical studies, patients 3-5 % of oxidized methionine on the particular methionine site during clinical studies.' It gives you a much better control, so you end up having a low overall risk if you manage to monitor that during clinical studies and process development. So it is actually very valuable for decreasing the risk of particular attributes, depending on which ones you select, for giving it to patients.

Now saying that, it was wasn't easy. It looks all great because of the results that we got, but this was not an easy journey, and we are still not there yet because we are just about to file it with the agency.

Advancing Highly Sensitive Analytical Technology

When you are start using these highly sensitive methods, you have really got to make sure you understand the robustness of your method. You have got to understand how reproducible it is. Simply because you can detect a thousand species doesn't mean you can detect it day after day, lot after lot, run after run. How rugged is it? Will you get the same answer if you run it on a Tuesday compared to when you run it on a Thursday?

Depending on what you have done to the machine itself you can get different results. What actually affects the results? What can affect the sensitivity of your method? Very importantly, what interferes?

We were running this method, and then all of a sudden, halfway through development, we kept getting this unusual profile that we couldn't understand that we kept getting out of nowhere. Would you believe it was somebody in the labs who just swapped from a clear Eppindorf tube to using ones that were colored? They were blue or pink or whatever because they were trying to sort out their samples. They said, well it is easy, I am going to put my oxidized samples in the red ones, and this in the blue ones and whatever. Well, in the few

minutes the material was sitting in those Eppindorfs, you managed to leach out a very small amount of color, some sort of dye thing, that impacted this method.

We spent around a month chasing around what the heck these extra peaks were. Because it doesn't say in your SOP, 'use Eppindorf color number whatever.' A simple change like that can have an impact. We went even further to actually specify the manufacturer of the Eppindorf tube we used, because we went across three different manufacturers and found you can get completely different profiles just from the plastic they use. These methods are that sensitive.

So you have got to be careful, because if you do this incorrectly and you are starting to use these methods for process characterization and lot release, you could be in a world of hurt if you don't understand what is going on. These are very sensitive methods.

You do want to identify what the species are. The machine can tell you, we think it is oxidation or deamidation depending on the algorithm, but you want to try and actually create those actual attributes and put them in there by purifying them out just to confirm what you are doing. We will get into data interpretation, and, of course, as I say that link to other methods. You really do need to correlate what you are seeing with existing methods that everybody understands.

So we do have criteria for evaluating a peptide or an attribute in the method, and I am not going to go through this. This is very specific for those of you who are mass-spec experts. But we have had to decide what the criteria are for the actual peptides that we are going to be able to analyze versus ones we don't and what the variability is for the ones that we believe are below the level of sensitivity or below the level of reproducibility. We came out with these sorts of specific attributes for the peptides themselves.

Now as you can imagine you can't do this with manual interpretation. It is just impossible with the kinds of chromatograms or whatever you want to call them that come out of this machine. It would contain almost, you know, five hundred different peaks that someone would have to be staring at to try and work out what is going on. So we had to rely on the vendor of the analysis program to help us with this.

So then you start getting in to the Part 11 business of making sure that everything is okay from a GMP perspective – that the algorithms they are using aren't hiding peaks or creating peaks that aren't there. That took us, I think, about two years to work with the vendor of this software to make sure that it was Part ll compliant and validatable per the GMPs.

So the advantage is that we get a ton of data of out this equipment. But the disadvantage is that we now have to make sure that we can convince regulators and our inspectors that the algorithm itself isn't doing something untoward. You know it is easy when you are looking at a gel to hand that to a regulator and they can see the bands on the gel and go, 'oh yeah, I can see that there is stuff going on.'

Here, we are just going to hand you a table that says, here is the amount of oxidation and here is the amount of deamidation. You are going to have to take it on faith that the software that we have is actually giving reasonable answers. Obviously, there will be packages you can stare at, validation packages, but it is not as easy as it would be for something that you can just visualize. That is the world that we are all going to have to get used to as we move to these sorts of technologies.

And, of course, the other issue is that we are going to get tons more data than we have ever seen before out of this method. What do you do with that? You compare it to mass-spec NMR analysis. How much of this data are you actually going to use? I believe that if we pick the relevant quality attributes, those are the bits that we are going to be looking at.

But as we have heard, regulators are somewhat conservative, and they are going to go, 'well are you looking at any of these other peaks that are floating around, and how are you going to control anything that goes up or down?'

When you have got 500 of them, are we going to have to work with 500 different peaks and work out how they wobble up and down from lot to lot and from day to day and all that sort of stuff? We are never going to be able to achieve that with these sorts of methods. So to a certain extent we are going to have to stick to the parts of the readout that these complex methods give that are relative to the quality attributes we are looking for.

Now hopefully we will be able to set some sort of threshold to say that if some peak trebles in size then all of a sudden the system will ping us. That actually took a long time to work with the vendors of the analysis material to get that. Rest assured, we cannot possibly provide control over every single peak that comes out of this analysis.

That is also something with the increasing technology and QbD understanding that we are going to have to get used to as an industry and regulators as we move forward.

MAM Qualification

- System suitability: Based on reference peak area, RT and S/N
- Specificity: Based on Mass accuracy, isotopic distribution, retention time
- Precision: Based on area % of peptide measured by MS extracted ion chromatogram. Includes repeatability and intermediate precision
- Accuracy: Based on comparison to theoretical mass
- Linearity: Based on area of peptide measured by MS extracted ion chromatogram
- LOD/LOQ: Based on quality of spectral data (TBD)
- Integrity limit: Still defining threshold peak detection parameters
- Robustness: At issue are conditions for sample prep, chromatography, and MS conditions

So the qualification is underway. You saw that we have already defined some useful system suitability criteria. We have looked at the specificity of the method to understand how it is actually working and how relevant it is from day to day. You have got precision…looking specifically at those attributes you want to look at. The accuracy: obviously we have done work to compare to theoretical mass, so when it tells us this is a deamidation of position x, it truly is a deamidation of position x.

We looked at linearity. That is always a useful check to make sure that as you dilute material that the machinery comes up with the relevant appropriate levels of the material. The LOD/LOQ, et cetera.

Robustness was a tough one because you are relying on people doing sample analysis. One day it would be lovely to be able to literally take a sample straight off the floor and put it into the machine. We are not there yet, but I am sure the technology will get there. We do have to do some sample prep. You have to make sure the chromatography conditions if you are using them are going to be appropriate, and especially the mass-spec conditions as you are using them.

The Regulatory Interface

So the path forward is, we are currently completing our correlation with existing methods [for] the products we want to apply this to and file it with. You do have to compare with the CEX, the size exclusion, and all the other methods you are going to be using – all the glycolization analysis, the traditional ones.

It is going to be a reasonable package that is going to be going into the agency. What we hope is that eventually as you all get used to it over the next two to three years, we wouldn't keep on having to do that. The fact is, if you show for five products, or five monoclonals, that every time we do it, it correlates well with the traditional methods, hopefully we can say goodbye to the traditional methods. Like with anything, it is going to take a while. It is a progressive movement.

Specification strategy: We are working on that now. It is not that easy, because of course we have never looked at these individual attributes before. It is almost gaining knowledge, particularly if you want to use legacy products from time zero, because you have to go back and get all that data again. But hopefully it shouldn't be so bad for new products as we move forward.

The validation strategy, as I said, wasn't easy. The validation of these methods, especially for something like mass-spec, is not easy, which is why it hasn't moved as rapidly into the QC world as we may have liked over the last few years. And of course, making sure that is ticks all the GMP Part 11 buckets.

What we are going to do is start applying these now in our regulatory filings. Our initial strategy is to file it as a characterization method, so that the agencies get used to seeing it side-by-side with our specifications. We decided it was too scary to have both run side by side in our specs. It causes complications if you are a global company like Amgen is.

You can imagine there are some forward-thinking agencies like the FDA and Europe or whatever. But you can imagine that sending a multi-attribute method to some of the regions that we may end up is going to just blow their minds. They are now having trouble enough with the normal methods that we use, let alone sending this. So we are going to start off with having it is a characterization method to get everybody feeling more comfortable with it. Then probably by the time it gets to licensure, we would replace the old methods with this single method moving forward.

Conclusion

So in conclusion, we believe we have the fundamentals to move to the single multi-attribute method on our specs. We have the automated quantification combining this Orbitrap technology and the dedicated software that allows us to do it in a QC environment. It is very flexible. It is amazing the amount of information you can get out of this machine that you can use for process development, for characterizing your product, as well as using it for lot release and stability.

We believe that it is science-based, because we are going to hopefully be able to move to basically presenting you critical quality attributes and actual measurements, rather than saying that they are buried within a peak. I do believe that you can see from the methods that scientifically it is superior to existing methods. It gives you much more information, much more quantitative information that I think is better for all of us and better for patients to control our products moving forward.

And at the end of the day, which our upper management love, it does reduce the cost of quality. Because you can replace probably six, seven, or eight methods with this one. And the reagents aren't particularly expensive, whereas they are for some of the methods we are replacing.

It is a universal method. So once jurisdictions around the world get used to this type of methodology, import testing becomes much easier when you are working with regulatory authorities that like to retest your material during development. It is a single method. It takes us a lot of time to transfer eight or nine methods around the world to multiple jurisdictions. We will probably end up paying for it and sending it to everybody, but once regulatory authorities have these mass-specs, it should be easier to transfer tests around the world.