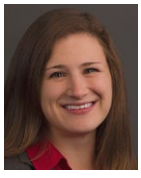
A Q&A

Critical Quality Attributes: The Ins and Outs of LC-Based Analytical Tools and Techniques



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The importance of critical quality attributes for the safety and efficacy of biotherapeutics.

At the 2018 WCBP Symposium in Washington, D.C.—a meeting intended to address issues at the intersection of analytical development and global regulations for biotherapeutics—much of the discussion throughout the week revolved around critical quality attributes (CQAs). Attendees wanted to know what are CQAs, how to define them, how to identify them, and how to monitor them. *BioPharm International* recently sat down with Anne Blackwell, PhD, an applications scientist at Agilent Technologies, to talk about these important issues, some of the analytical challenges scientists face, and new tools available to meet those challenges.

BioPharm International: What makes CQAs such a hot topic for biotherapeutics?
Blackwell: Ultimately, it boils down to the safety and efficacy of the drug product, the importance of which cannot be understated. CQAs are all the properties of a biomolecule that may affect how safe and effective it is. One aspect of this inspection is identifying and defining the CQAs. For example, which structural properties of the protein have an impact on its safety or efficacy, and which do not? Another aspect is the huge analytical challenge of measuring all of these attributes with sufficient sensitivity, resolution, speed, and accuracy.

BioPharm International: Can you give an example demonstrating the impact of a CQA on safety, and how one might monitor that CQA?
Blackwell: Aggregation provides a clear example of this. Aggregation of a biotherapeutic can trigger an undesirable immune response. Unfortunately, aggregation is fairly common. When a protein is stressed by sample handling or storage conditions, for example, it can aggregate.

Size-exclusion chromatography (SEC) is the go-to approach for measuring aggregation. Agilent's AdvanceBio SEC columns are a great tool for this. The inert chemistry of the packing material minimizes secondary interactions. As a result, it is not necessary to add extra salt or organic solvents to the mobile phase, which could influence the accuracy of measurements. Either of these additives could inadvertently affect the aggregation state of the protein. Ideally, the measurement should reflect what is present without changing what is present.

BioPharm International: Could you please provide an example of how LC and other techniques can be used together to form a more complete analysis of the CQA?
Blackwell: In terms of aggregation analysis, SEC is really good at measuring the relative abundance of monomer to dimer. It can provide insights regarding sediments or higher-order

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aggregates. An SEC run can take roughly 15 minutes, however, and the sample is tough to recover.

In a high-throughput environment, it may make sense to use a faster screen first. A UV-Vis screen could be used initially to quickly determine the relative amount of aggregate. UV-Vis can provide that measurement within seconds. Then, any samples that need further quantitative analysis of the aggregates could be flagged for SEC.

Even more information can be obtained by combining SEC with a light-scattering detector. With light scattering, it is possible to determine the molecular weight and then confirm the aggregation state of the protein. So, certain tools can be used either before or in addition to SEC to flesh out more information.

BioPharm International: What is one way that scientists can try to control these CQAs, perhaps by limiting product-related impurities or by encouraging the production of a single specific protein product?

Blackwell: Risk is reduced if a uniform product can be produced in the first place. This can also simplify the downstream purification and analysis process. Carefully optimizing and monitoring the cell culture media conditions in which the biotherapeutic is produced can lead to a more uniform product.

Every protein and biotherapeutic is unique. But ensuring that the cells have everything they need—and only what they need—can lead to fewer sequence variants or even to glycosylation patterns that are more uniform. The UV detection of derivatized amino acids (e.g., by using a tool such as AdvanceBio Amino Acid Analysis kit) has been a common approach to this for a long time. Recently, in addition to looking at amino acids, there has been increasing interest in considering what other components of the media are key to productivity. Moreover, by determining which compounds in the spent media are indicative of a problem, one can quickly address and remedy the issue.

Agilent's newest column is a great tool for this. The AdvanceBio MS Spent Media column with mass spectrometry detection allows the analysis of underivatized amino acids in samples. But it is also possible to look at other cell culture metabolites at the same time, using a single analytical method.

BioPharm International: Multi-attribute methods sound like an exciting, but challenging, means of monitoring CQAs. Please describe those challenges and what one might look for in a column and chromatography technique for multi-attribute methods.

Blackwell: Multi-attribute methods are definitely a challenge, but a really interesting one. All analyses cannot be replaced with a single method. However, in addition to the primary sequence of a protein, a single method can potentially monitor a wide variety of post-translational modifications, ranging from clips to deamidation, or even glycosylation.

Multi-attribute methods rely upon the specificity of mass spectrometry detection. Not only does it determine that there was a change, but it also identifies exactly what that change is. Analysts will want columns that enable them to take full advantage of the sensitivity and specificity of mass spectrometry such as having sharp, symmetric peaks under formic acid mobile phase conditions.

Furthermore, the goal of multi-attribute methods is to simultaneously monitor a wide variety of CQAs. The ability to separate a range of peptides and to resolve post-translational modifications, especially isomers, is key. AdvanceBio Peptide Mapping and AdvanceBio Peptide Plus are two reversed-phase columns that have complementary selectivity and retention for peptide mapping and multi-attribute methods.

BioPharm International: Many of the columns you have mentioned include AdvanceBio in the name. What is the driver behind the AdvanceBio branding?

Blackwell: The AdvanceBio brand reflects Agilent's effort to design column products specifically to address the challenges of analyzing biological molecules. Agilent has been in the small-molecule chromatography market for a long time, but biologics definitely have unique challenges versus small molecules.

Therefore, Agilent is consciously testing columns on these applications in the research and development stage, as well as through application-specific QA testing during column production. Agilent is working hard to expand its AdvanceBio portfolio to offer all the separation tools needed to fully characterize biotherapeutics, to accurately monitor CQAs, and to back that portfolio with expert support.

Agilent's goal is to provide its customers with the tools they need to be successful. That requires us to listen carefully to what they need and then innovate to create those solutions.