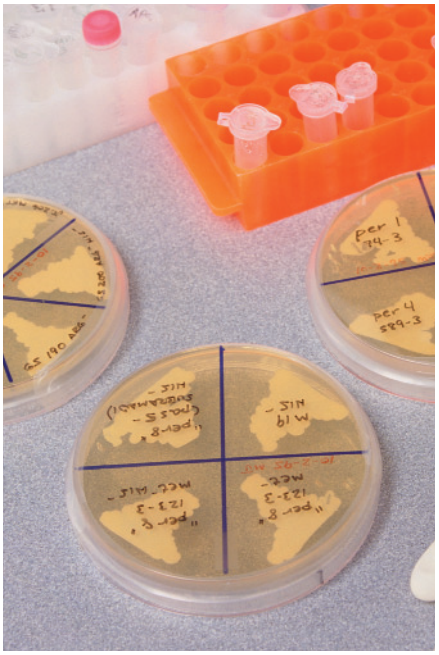

Using Peptide Maps as Identity and Purity Tests for Lot Release Testing of Recombinant Therapeutic Proteins

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FDA and biopharmaceutical industry stakeholders reach consensus on critical issues related to the use of peptide maps as identity tests for proteins and on their usefulness for lot release tests. The results of that discussion are presented here.

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Peptide mapping plays an important role in the ensemble of analytical tools that are used for lot release testing of recombinant therapeutic proteins. Peptide maps are primarily used to establish product identity by confirming the primary structure (the amino acid sequence) of a product on a lot-to-lot basis. Peptide mapping has also been used as a nonregulatory test. Peptide mapping is also endorsed as an acceptable test for establishing the genetic stability of a product-producing organism throughout the life cycle of the product (1,2).

Numerous publications have described the development and validation of peptide maps for a variety of recombinant protein therapeutics (3–5). These studies reinforce the acceptability of using peptide maps as tests for lot release or identity tests, nonregulatory purity evaluations, and genetic stability assurance. In general, the published studies demonstrate that — when appropriately developed and validated — peptide mapping is robust, precise, and capable of detecting single amino acid substitutions or side-chain modifications with statistical confidence.

Specifications for Peptide Maps

When a peptide map is used as a lot release identity test, the acceptance criterion for the specification is usually confined to statements like "Compares to Reference" or something similar. Given the complexity of the test and the large amount of information obtained, appropriate method acceptance criteria are critical to ensuring the validity of the lot release test results. And reducing the information so that the test result of a given lot can be directly compared to the specified acceptance criterion requires appropriate methods as well. It is within this context that manufacturers struggle to employ a consistent approach for comparing lot release test results. The method acceptance criteria also enable the direct comparison of the peptide map test results to the specification. No regulatory guidance has yet been published to help in developing consistent comparison methods.

A Strategy Forum

The first Well-Characterized Biotechnology Pharmaceutical (WCBP) Chemistry Manufacturing and Controls (CMC) Strategy Forum was held on 19 September 2002, in Boston to discuss these specific peptide mapping issues. The California Separation Science Society (CaSSS) sponsored the forum. For a description of the WCBP CMC Strategy Forum as well as information on CaSSS and some of its other meetings, see the "Industry–FDA Collaborate on CMC Concerns and Other Biotechnology Issues."

The objective of the meeting was twofold: Address critical issues related to the use of peptide maps as an identity test for proteins, and obtain consensus on using a peptide map as a release test. Two roundtable discussions were held to provide a forum for further discussion. The first roundtable was "Is Visual Comparison of a Peptide Map to a Reference Standard Peptide Map an Appropriate Criterion (for an Identity Test) for Release Testing," and the second discussion was entitled "Can/Should Release Testing of Proteins be Performed Without Using Peptide Maps."

This article provides a guideline for using peptide maps based on the consensus reached among the delegates at the forum.

Peptide Maps and Standards

As stated in the International Conference on Harmonisation (ICH) Q6B guideline on biotechnological substances, an identity test

should be highly specific for the drug substance or product and should be based on unique aspects of its molecular structure or other specific properties (6).

A peptide map of a protein provides the specificity needed for an identity test. However, the appropriate criteria to confirm the identity of a protein based on a peptide map need to be determined. Is it essential to perform a relative quantitation of the peak areas (from the peptide map) for a reference standard and for a product sample? Or is a visual comparison of the reference standard and sample peptide maps appropriate to confirm the identity of a protein?

Consensus at the forum was that:

- Visual comparison of the peptide map to the reference standard is an acceptable criterion to assess the identity of a protein. Visual comparison of the number and relative intensity of the various peptides in a peptide map should provide adequate information about the identity of a protein.
- The peptide maps of the sample and the reference can be overlaid to evaluate the presence of all peaks at appropriate retention times and signal intensity.
- Generation of peptide maps from a 50:50 comixture of the sample and the reference is recommended to account for minor variability in the retention time of the peptides.

In addition to those criteria, appropriate system suitability and method acceptance criteria should be met.

Significant new peaks. At issue with the use of peptide mapping as an identity test is the appearance of significant new peaks in the chromatogram. These peaks may result from degradation or from other modifications of the protein. Consensus at the forum on this issue included the following agreements.

- To ignore new peaks in the peptide map is not justifiable.
- Consideration of additional peaks in the peptide map of a sample compared to the reference standard map moves the test into the realm of a purity test. As a result, continued distinction of the peptide map as an identity test as opposed to a purity test must be maintained.
- The appearance of a significant new peak in the map does not, per se, result in failure to meet an appropriate identity specification. The appearance of the new peak should trigger an investigation as part of the test procedure that is not tied to the specification.

Industry–FDA Collaborate on CMC Concerns and Other Biotechnology Issues

The Well-Characterized Biotechnology Pharmaceutical (WCBP) Chemistry Manufacturing and Controls (CMC) Strategy Forum was created to provide a venue for discussing state-of-the-art innovations and conventional technologies, which are used in biotechnology product applications. The forum is meant to do the following.

- Focus on industry and FDA CMC concerns.
- Foster collaborative technical and regulatory interactions that arrive at consensus for everyone's mutual benefit.
- Provide FDA with information that will enable it to merge good scientific practices with good regulatory practices.

The first WCBP CMC Strategy Forum was attended by 55 participants. The case studies, which participants said provided an appropriate spectrum of examples, were well received and sparked considerable discussion.

The California Separation Science Society (CaSSS) is the primary sponsor of the forum. CaSSS holds three one-day workshops in Bethesda/Rockville, Philadelphia, Boston, and San Francisco each year, which focus on a maximum of two topics, and that consist of formal presentations by industry or FDA to introduce topics of concern.

The CaSSS website (www.casss.org) has online versions of the various presentations made at its forums and workshops.

Additional CaSSS conferences. The second WCBP CMC strategy meeting was held 6 January 2003, and focused on the analysis and structure characterization of monoclonal antibodies. Next year's CMC Strategy Forum will precede the WCBP 2004 conference in Washington DC on 5–9 January (for both the forum and the conference). The conference will focus on the interface of regulatory and analytical sciences for biotechnology health products.

CaSSS also sponsors the annual FDA Science Forum, now in its 9th year. This year's Science Forum, "FDA Science: Protecting America's Health," will be 24–25 April in Washington DC. Other sponsors include FDA, Williamsburg BioProcessing Foundation, and AOAC International.

The FDA Science Forum is a comprehensive training program to communicate and promote issues relating to scientific development and associated regulatory concerns. FDA uses this forum to award employees for scientific achievement and to showcase its scientific achievements as well as to present topics of interest and promote collaboration. The forum is designed to bring together scientists from FDA, industry, academia, other government agencies and consumer and patient advocacy groups, Congress, and international stakeholders.

An online discussion. CaSSS has set up a CMC electronic discussion list as a communication forum for anyone involved in the development of technical and regulatory consensus. The online discussion group will facilitate reviews of position papers and discuss future CMC issues.

The online forum will also provide a venue for discussing the latest biomolecular methods and their practical application to biotechnology pharmaceuticals, which includes both method and instrumental advances that are used for product characterization, process development, and validated in-process, release, and stability tests. Requests for help on particular problems may be posted to the list as well. The list can also be used to announce meetings, call for papers, and other items of interest. Commercial postings and personal messages are not allowed.

- For those peptide maps that use ultraviolet (UV) absorbance detection, a peak response threshold can be established to define a significant new peak based on method validation experience. New peaks that exceed that threshold result in a failure of the test article to meet the specification.

Peptide Maps or Other Tests?

Although peptide maps are routinely used to assess the identity and purity of a protein, it may be worth considering a battery of other, simpler, analytical tests.

- Analytical tests such as isoelectric focusing (IEF) and high performance liquid chromatography (HPLC) are sufficiently specific to establish identity of a protein.
- Although the chromatographic profiles of antibodies are similar, subtle structural differences can be used to differentiate between the various antibodies. Capillary isoelectric focusing (cIEF, capillary electrophoresis) or ion-exchange chromatography may provide the required specificity for an identity test.
- Bioassays are a good choice as an identity test. The results of a bioassay used to demonstrate potency can also be used as an identity test. For antibodies, a potency test provides a quantitative measure of an antibody's binding to its receptor — and that ability to bind to a specific receptor can be used to identify the antibody.

Peptide maps as security blankets. If a release testing regime doesn't include a peptide map, will the testing regime still detect something "out of the ordinary" that results from genetic instability in the cells used to express the protein or from process contaminants? Should the peptide map be viewed as a security blanket?

The consensus at the CMC Forum was that variants generated by the genetic instability of cells should not be an issue, particularly during the later stages of process development. The experience gained throughout the product's development cycle will provide a greater understanding of the cell culture process and the stability of the cells, ruling out genetic instability as an issue.

Additional recommendations from the CMC Forum included:

- Extensive characterization of a protein should be performed (peptide mapping and LC/MS analysis) during the development cycle and during the process validation stages to rule out issues with genetic instability and process variability.
- Peptide mapping is advised for release testing of batches produced before the manufacturing process is validated.
- A peptide map is a powerful tool to determine the purity of a protein whether that entails assessing the level of oxidation, deamidation, or degradation. Protein purity can also be determined by a variety of chromatographic and electrophoretic methods.

Use with Consideration

The role of a peptide map in quality control is product specific and should be considered within the context of all the analytical tests used to control the product attributes as well as the clinical experience obtained during product development. All things considered, the argument is plausible that a peptide map may not be needed as a lot release test as another or a combination of other analytical tests can suffice to appropriately establish product identity or purity. ***BPI***

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