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An FDA perspective on the implementation of state-of-the-art analytical methods for therapeutic proteins

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FDA

An FDA Perspective on the Implementation of State-of-the-Art Analytical Methods for Therapeutic Proteins

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Manufacturing Innovation for Biologics
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Disclaimer

- The views and opinions expressed in this presentation belong to me and do not represent official FDA policy.

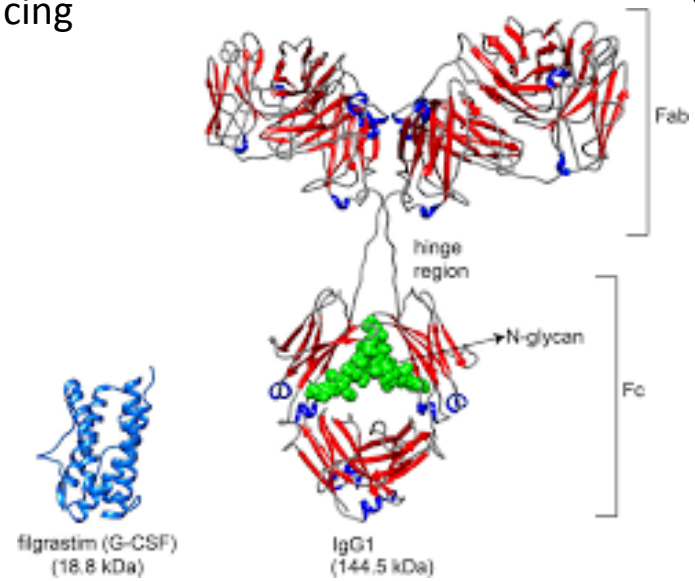
Outline

- Evolution of analytical tool box
  - Mass Spec
  - CE
- State-of-the-Art analytical methods through the product lifecycle
  - Expectations
- FDA's Emerging Technology Program
  - Small molecule examples
  - Potential application to and challenges for biotech products
- Take home messages

1990s Analytical Tool Box

1º Sequence/PTMs
AA analysis
N- and C-term Sequence
Peptide Mapping and Sequencing
LC-MS/MS (1 sponsor)
MALDI-TOF (BLA)
ESI-MS (BLA)

HOS
CD (1 sponsor)
DSC (BLA)



Japelj et al Sci Reports 2016

Glycan Analysis
Monosaccharide analysis
CE with fluorescence detection (BLA)

Charge/Identity
IEF
IEX
cIEF

Process Related Impurities
Largely focused on bovine proteins
BSA, transferrin, IgG

Size/ Purity
SEC-HPLC
SDS-PAGE R + NR
Coomassie Blue and Silver Stain
Immunoblotting
CGE (BLA)

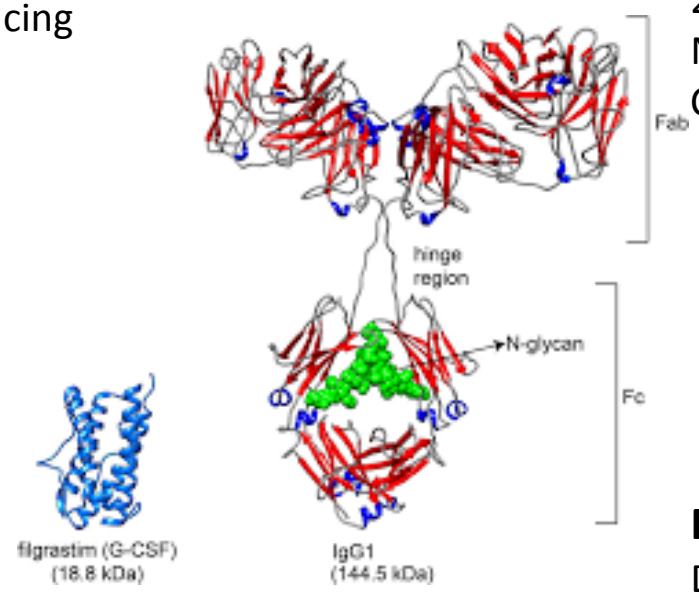
Activity
In vitro/ in vivo Bioassays
Binding ELISAs
Flow cytometry
Strength (UV A280)
BCA (1 DS)

Safety
Bioburden
Sterility
Rabbit Pyrogens
Endotoxin
General Safety

2000s Analytical Tool Box

1º Sequence/PTMs
AA analysis
N- and C-term Sequence
Peptide Mapping and Sequencing
  LC-MS/MS
MALDI-TOF
ESI- MS
QTOF
Ion trap

HOS
CD
Fluorescence spec



Japelj et al Sci Reports 2016

Glycan Analysis
Monosaccharide analysis
2-AB Labeled, PNGaseF released
NP-HPLC
CE-LIF

Charge
IEF
IEX- HPLC
CEX
cIEF

Process Related Impurities
DNA, HCP, Protein A, etc.

Size/ Purity
SEC-HPLC
SDS-PAGE R + NR
  Coomassie Blue and
  Silver Stain
Immunoblotting
CE-SDS/CGE

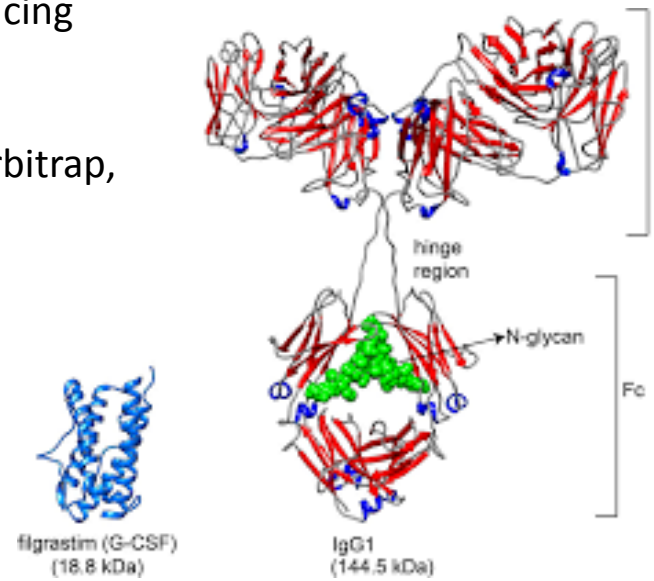
Activity
In vitro Bioassays
Ag/Receptor Binding assays
Flow cytometry
SPR
Strength (UV A280)

Safety
Bioburden
Sterility
Endotoxin
  LAL

The Current Analytical Tool Box

1º Sequence/PTMs
AA analysis
N- and C-term Sequence
Peptide Mapping and Sequencing
  LC-MS/MS
Free sulfhydryls
MALDI-TOF, ESI-QTOF-MS, orbitrap, etc….

HOS
Near- and Far-UV CD
FTIR
DSC
HDX-MS
X-ray
NMR



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Size/ Purity
SEC-HPLC
HIC-HPLC
CE-SDS
CGE
AUC
A4F

Activity
In vitro Bioassays
  Reporter gene assays
Ag/Receptor Binding assays
  (mAbs – FcR, C1q)
SPR
Strength (UV A280)

Glycan Analysis
ESI- MS
MALDI-TOF MS
Labeled, PNGaseF released
  HPAEC-PAD
  HPLC-FD
  HILIC (HPLC, UHPLC)
  UPHPLC
  CE-LIF (MS)

Charge
cIEF
icIEF
ICE
IEX- HPLC
CZE

Process Related Impurities
DNA, HCP, Protein A, etc.

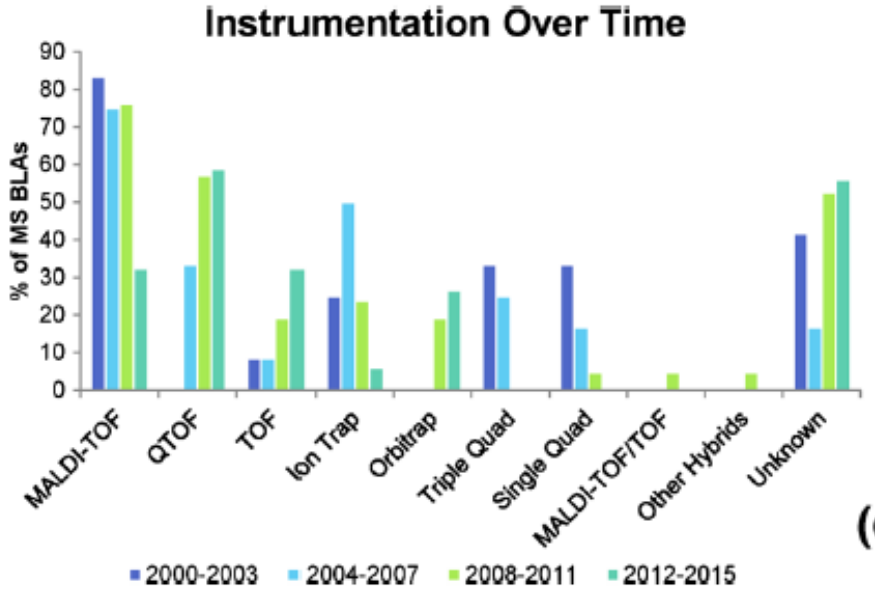
Safety
Bioburden
Sterility
Endotoxin
  LAL
  KT

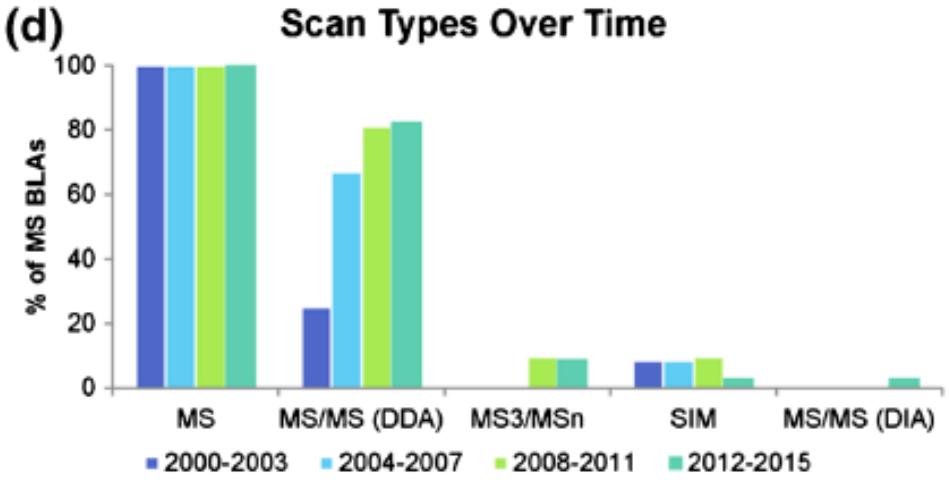
A Retrospective Evaluation of the Use of Mass Spectrometry in FDA Biologics License Applications

- 79/80 electronic submission BLA between 2000 and 2015 used MS for characterization
  - mAbs, ADCs, fusion-proteins, other proteins
- 32 specific attributes were analyzed
- Trends were noted for MS work flows, methods, instrumentation, and attributes analyzed over time
- "…we expect that we will see additional MS methodology within the quality control and comparability sections."

Rogstad, S. et al., J. Am. Soc. Mass Spectrom. (2016)

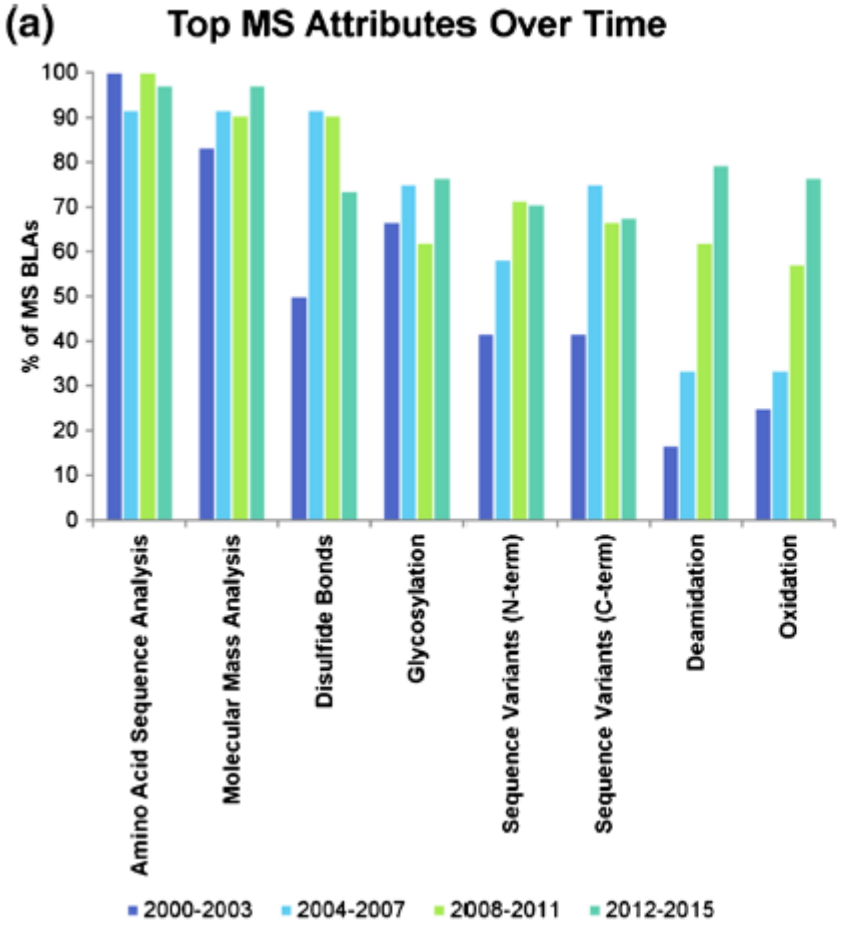
Introduction of MS Instruments and Scan Types Over Time

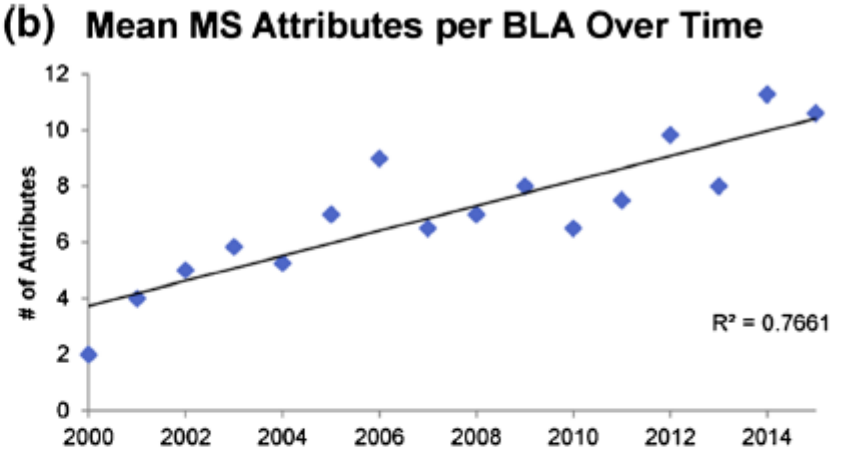


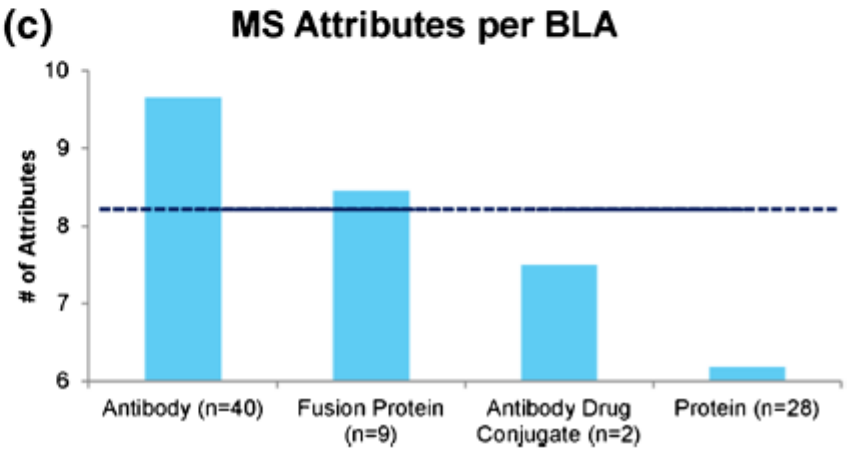


Rogstad, S. et al., J. Am. Soc. Mass Spectrom. (2016)

Major MS Attributes for Analysis







Rogstad, S. et al., J. Am. Soc. Mass Spectrom. (2016)

It Takes Time for New Methods to be Used Routinely for QC

- Although we saw some CE based methods for release/stability in the late 1990s, they became "routine" in the past 5-10 years
- CE method(s) are included in the specs for:
  - 35% of products through 2009
  - 44% of products through October 2014
    - 58% of products approved in the 5 years prior to the 2014 meeting
  - 52% of products up to September 2016
    - 90% of products approved in the 2 years since the 2014 meeting

Source: Sarah Kennett CASSS CE Pharm meeting

State-of-the-Art Analytical Methods Throughout the Product Lifecycle

R&D Pre-clinical

High throughput methods, NGS, MAM, metabolomics, PCA for

- Candidate Selection
- Cell line development
- Process Development

Phase 1 Phase 2 Phase 3

Regulatory expectations

- Characterization (SotA)
- Robust methods for release and stability
- Update methods and panel of methods as appropriate for release, stability, characterization and comparability

Comparability Analytical Method Lifecycle

Regulatory expectations

- Characterization (SotA)
- Robust methods for release and stability
- Update methods and panel of methods as appropriate
- **OK if updated methods find new things that were always there, resulting in a change in specs**

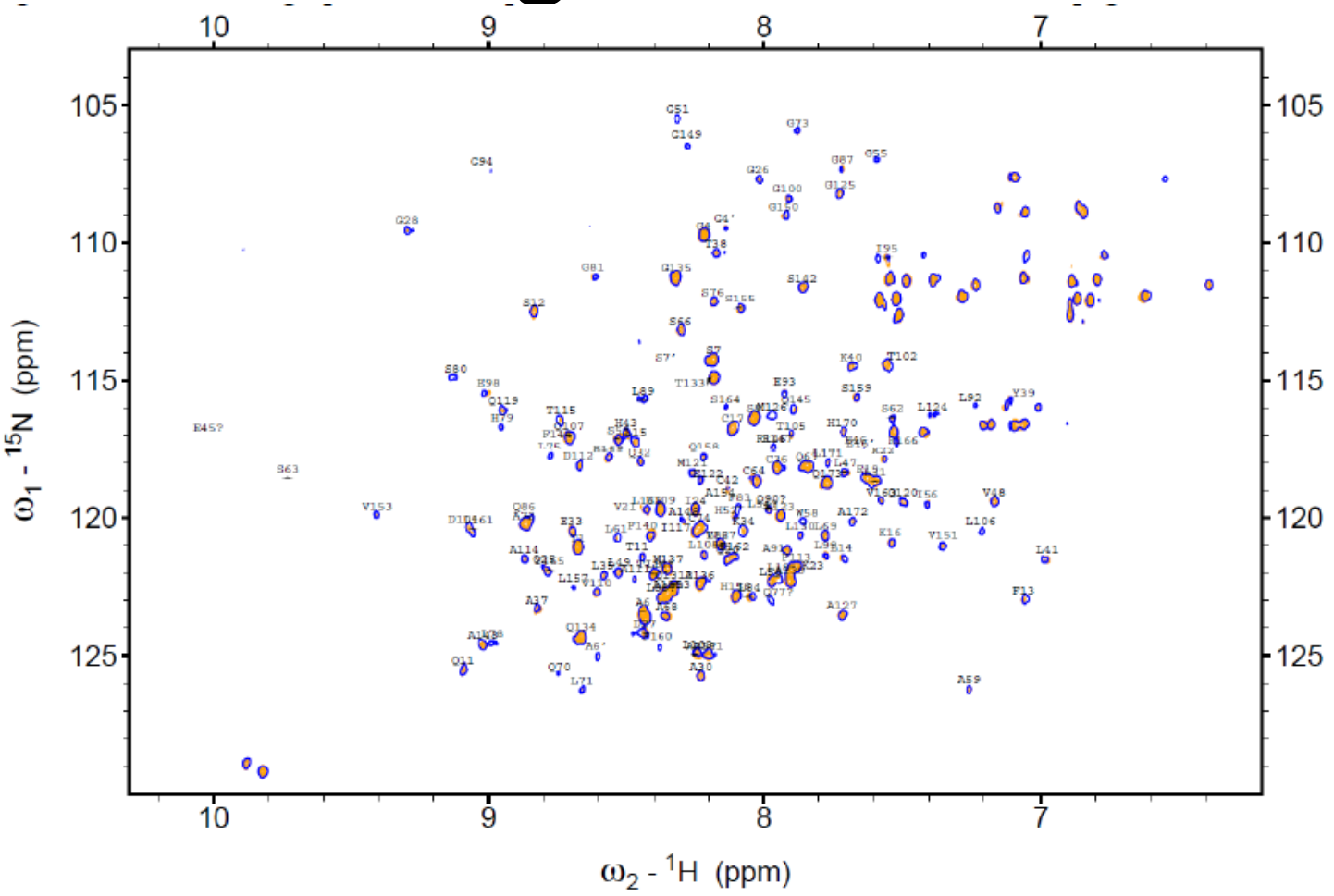
Impact of Biosimilars

Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product

- Sponsors should use appropriate analyti… dequate sensitivity and specificity to detect… veen the proposed product and the ref… rages the use of widely availab…
- A meaningful a… similar to … ca… ess, for … the protein … tions), degree of h… ofiles, and degradation pr… the methods used in these ana… limitations, should be described by the …
- Curre… nology is capable of evaluating the three-dimensional structu… proteins. Using multiple, relevant, **state-of-the-art method**… help define tertiary protein structure and, to varying extents, quaterna… ructure and can add to the body of information supporting biosimilarity.

If we expect biosimilar Sponsors to do this, should we have the same expectations for all Sponsors?

2D NMR of Filgrastim



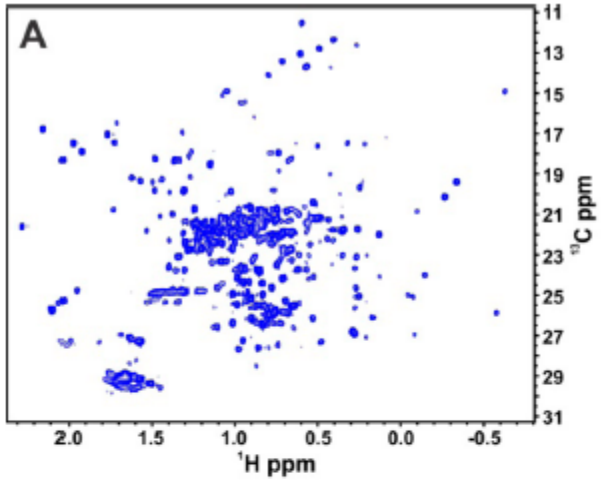
US- licensed Neupogen batch (orange) and one ZARXIO batch (blue)

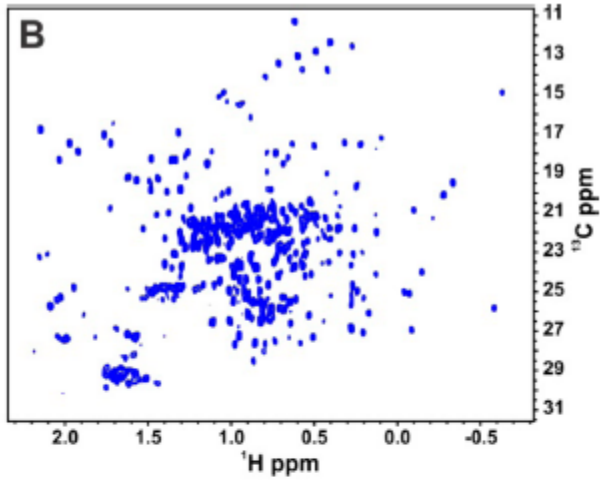
Figure from Sandoz ODAC briefing package 1/7/2015

2D NMR of NIST mAb

Could be used for comparability – but is it value added?

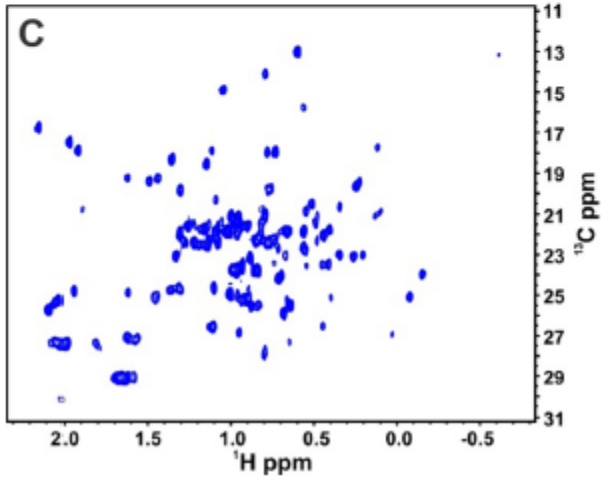
Intact mAb

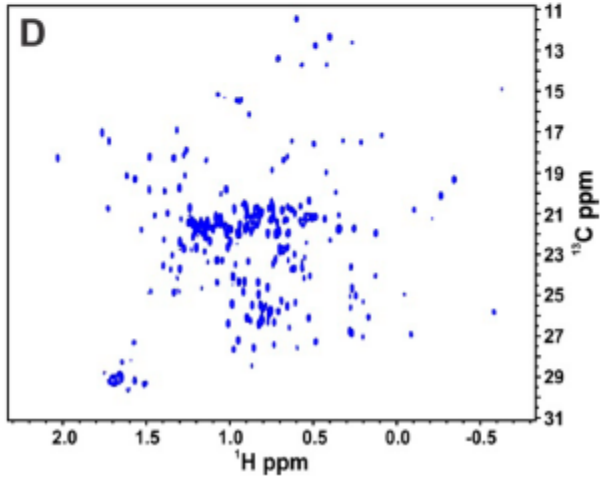




Fab + Fc

Fc fragment





Fab Fragment

Arbogast et al. Analytical Chemistry 87: 3556, 2015

It Depends…..

Methods seen more often in biosimilar packages

Mostly HOS methods

- HDX-MS
- NMR (1D and 2D)
- X-ray crystallography

Multiple MOA methods

- Some MOAs may not have been known or understood at the time the reference product was licensed, or good methods were not available.

Many methods are standard across sponsors

- Capillary based methods (size and charge)
- Multiple MS methods for sequencing, PTM identification/quantitation, glycan analysis
- Glycan profiling
- Other HOS methods (CD, FTIR, DSC)
- Size methods (SEC, AUC, SEC-MALLS)
- SVP analysis (HIAC, MFI, Archimedes)
- Methods that assess biological function
  - Bioassays
  - Immunochemical/biochemical assays
  - Binding assays

State of the Art Methods

- Used first as characterization methods
  - Are not validated, but fit for purpose
  - May not be readily transferable and may require specialists
  - As seen for capillary based methods, it took a while for routine use in QC labs
- MS methods may not be practical for QC
  - New methods and instruments introduced often
  - Need an instrument and software that vendor will support for many years
- Which HOS methods are best suited for comparability and/or analytical similarity of mAbs?
  - Can you tell one IgG1 apart from another?
- But could be invaluable for understanding the process and product during development
  - Fit for purpose

Emerging Technology Draft Guidance (2015)

- Modernizing manufacturing technology may lead to a more robust manufacturing process with fewer interruptions in production, fewer product failures (before or after distribution), and greater assurance that the drug products manufactured in any given period of time will provide the expected clinical performance

- Examples of such elements in a planned submission include an innovative or novel: (1) product manufacturing technology, such as the dosage form; (2) manufacturing process (e.g., design, scale-up, and/or commercial scale); and/or (3) testing technology

- Will generally be unfamiliar in both industrial and regulatory contexts with limited or no regulatory precedence

https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM478821.pdf

FDA's Emerging Technology Program

- Experience with Small Molecule Drugs
  - 3-D Printed Tablet (way cool!)
    - Approved in 2015
    - Rapidly disintegrating, easy to swallow
  - Novel Long-Acting Oral Drug Delivery
    - Current extended and sustained release achieves therapeutic serum levels for 12-24 hours
    - Aim to extend this out to >1 week with 1 pill
    - Will improve adherence to medication regimens
  - Continuous Manufacturing
    - Approved a PAS for oral tablets

Contacting the Emerging Technology Team (ETT)

- ETT Contact: CDER-ETT@fda.hhs.gov
- Early Stage of Development: ET proposal may or may not be tied to a particular product or regulatory submission
- Advanced Stage of Development: Pre-submission meetings for regulatory applications with ET component (INDs, BLAs)

Where is Biotech Headed?

- Multi-Attribute Methods
  - Mass Spectrometry
    - Bottom up
    - Top down
    - Middle out
- Continuous Manufacturing
  - Advanced Process Controls
    - PAT
    - RTRT
- Already exist to some extent for biotech products

Mass Spec Based Multi-Attribute Methods

- Mass Spec played an important role in thinking of therapeutic proteins as “well characterized”.
- MS can be coupled with separation technologies.
  - MS can identify and quantify specific PTMs and sequence variants and when coupled with separation techniques, can tell you which peak contains the variant.

But…

- Can MS replace QC methods such as CE, IEX, SEC, RP-HPLC and HIC-HPLC, which tell you about quality attributes of the population, but not at a molecular level?
- Can MS be used to move release testing to in-process testing?

Considerations/Concerns

- Some sample preparation steps can alter specific QAs.
- Bottom up approaches may not be/are not sufficient.
- Are you analyzing the correct attributes?
- You've identified and quantified specific PTMs and sequence variants, but do you know if they are evenly distributed across molecules or only on 10% of the population?

Considerations/Concerns

- If the PTM has the potential to affect potency or activity, does knowing the overall level tell you what you need to know?
  - For example, if CDRs of a mAb may be prone to 2 PTMs, is one PTM sufficient to reduce potency or would both PTMs be needed, on one or both halves of the molecule ?
  - May not be able to tell you if there was an overall shift in the PI of the product, which could affect PK of sc administration
  - However, may be better for setting a spec around a specific PTM with a known impact, rather than setting a spec on an acidic or basic peak.
- If you want to use MS for in-process testing instead of release testing, are you using it in the correct place during manufacture?
  - Can the attributes you are assessing be affected by steps downstream of where you are testing?
- Have you performed an adequate risk assessment of the testing strategy on potency, PK, safety and immunogenicity?
  - Does the MAM give you the information you/we need in order to make appropriate decisions?

Continuous Manufacturing

- Perfusion bioreactors
  - Some approved products are manufactured in perfusion bioreactors followed by batch downstream processes
  - Can be run at high cell densities resulting in higher productivity with reduced impurities in the harvest
  - Need stable cell lines and optimal media formulations that can produce consistent yields for prolonged periods.
  - Could minimize PTMs and degradation that are associated with prolonged exposure to bioreactor pH and temperature or host cell proteolytic enzymes.
- Downstream single use process technologies
  - Some experience with continuous downstream processes
    - Not for entire downstream process
  - Challenges for multiple columns, continuous virus inactivation and UF/DF are being addressed.
  - Need to control bioburden over prolonged periods.

Konstantinov and Cooney. White Paper. J. Pharm. Sci 104:813. 2015

Continuous Manufacturing – Additional Challenges

- Need appropriate analytics at the right places
- Need good Advanced Process Controls for in-process tests and assurance of virus inactivation/removal
- Increased dependence on quality and consistency of raw materials
- Greater dependency of vendors of single use technologies
- For biotech products, the know-how exists
  - The challenges may not be regulatory

Konstantinov and Cooney. White Paper. J. Pharm. Sci 104:813. 2015

Take Home Messages

- Be innovative and push the envelope, but…
- Don’t oversell!
- Your new analytics/advanced technologies may be the greatest invention since sliced bread, but we need to come to the same conclusion (and we might not!)
- Put yourself in our shoes – what would be our concerns?
- Back up your claims with the right kind of data!
- Know your protein!

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