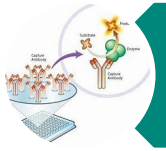
Current Thoughts on Bioanalytical Method Validation for Biotherapeutics by Mass Spectrometry

November 19, 2020

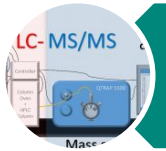
Faye Vazvaei & Eric Woolf

Bioanalytical Assays for Biotherapeutics

Ligand binding assays

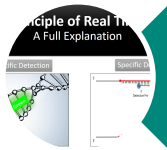


Enzymatic activity assays



Mass spectrometry assays

Affinity capture/mass spec assays



Other methodologies (RTPCR, Flow Cytometry, etc.)

Bioanalytical Strategies using Mass Spec

Top-down or intact analysis

- Direct measurement
- Preserves the structure
- Generally suitable for MW of <~10KDa (~60 KDa have been reported)
- Lacks sensitivity

Bottom-up or analysis using a surrogate peptide

- By far the most common method for PK
- Sensitive
- The structural information is lost – critical for characterization

Middle-down – analysis of subunits (not a lot of experience)

- Somewhat preserves the structure
- More sensitive than the intact

Which Method to be Used

LBA is the gold standard for protein bioanalysis and most widely utilized

Mass Spec is another tool in the tool-box

Depending on the molecule and the questions being asked Mass Spectrometry may be utilized

- Simplified method development – potentially less dependent on reagents
- Mass spec assays can help to overcome certain assay interference issues
- Multiplexed assays
- Evaluate *in vivo* structural modification, including deamidation/oxidation, biotransformation, etc.
- Other reasons

Method Validation—Why & How?

Why Should We Validate Methods

- The purpose of validation is to ensure that the quality of data generated by an assay is fit for purpose
- For PK we want to make sure that the variability of the assay is below that associated with day to day physiological variability

How should we validate methods

- We have the guidance documents (next few slides)

Why should we set acceptance Criteria

- To ensure that the assay is performing in a consistent manner; thus ensuring that it continues to deliver fit for purpose data

How do we set the acceptance criteria

- Fixed acceptance criteria, vs.
- Statistical Approach (e.g., determination of cut-point in ADA assays)?

Bioanalytical Method Validation Guidance

FDA Encourages Innovation

BMV Guidance, May 2018

"The FDA encourages the development and use of new bioanalytical technologies. However, the use of two different bioanalytical technologies for the development of a drug may generate data for the same product that could be difficult to interpret. This outcome can occur when one platform generates drug concentrations that differ from another platform. Therefore, when a new platform is used in the development of a drug, the data it produces should be bridged to that of the other method."

"The recommendations can be modified with justification, depending on the specific type of bioanalytical method. This guidance reflects advances in science and technology related to validating bioanalytical methods."

"In cases where one method produces data with a constant bias relative to the other, concentrations can be mathematically transformed by that factor to allow for appropriate study interpretation. Sponsors are encouraged to seek feedback."

But, no direct recommendations for hybrid assays or LC-MS for LM…

ICH M10 Draft

No Reference to LC-MS for Biotherapeutics

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

BIOANALYTICAL METHOD VALIDATION
M10

Draft version

Endorsed on 26 February 2019

EMA PK Guideline

The most frequently used analytical methods for assaying therapeutic proteins in biological samples are i) immunoassays, which estimate the amount of test compound that binds to a target antibody, and ii) bioassays, which measure the activity of the compound in a specific biological process.

…

Other methodologies, such as liquid chromatography-mass spectrometry (LC-MS), may be used but are not specifically addressed here. If possible, it is preferable to develop a specific assay early in the development and use the same assay(s) during the entire development program.

European Medicines Agency

London, 24 January 2007
Doc. Ref. CHMP/EWP/89249/2004

COMMITTEE FOR MEDICINAL PRODUCS FOR HUMAN USE (CHMP)

GUIDELINE ON THE CLINICAL INVESTIGATION OF THE PHARMACOKINETICS OF THERAPEUTIC PROTEINS

DRAFT AGREED BY THE EFFICACY WORKING PARTY	20 May 2005
ADOPTION BY CHMPFOR RELEASE FOR CONSULTATION	28 July 2005
END OF CONSULTATION (DEADLINE FOR COMMENTS)	28 January 2006
AGREED BY THE EFFICACY WORKING PARTY	9 January 2007
ADOPTION BY CHMP	24 January 2007
DATE FOR COMING INTO EFFECT	31 July 2007

AAPS Survey

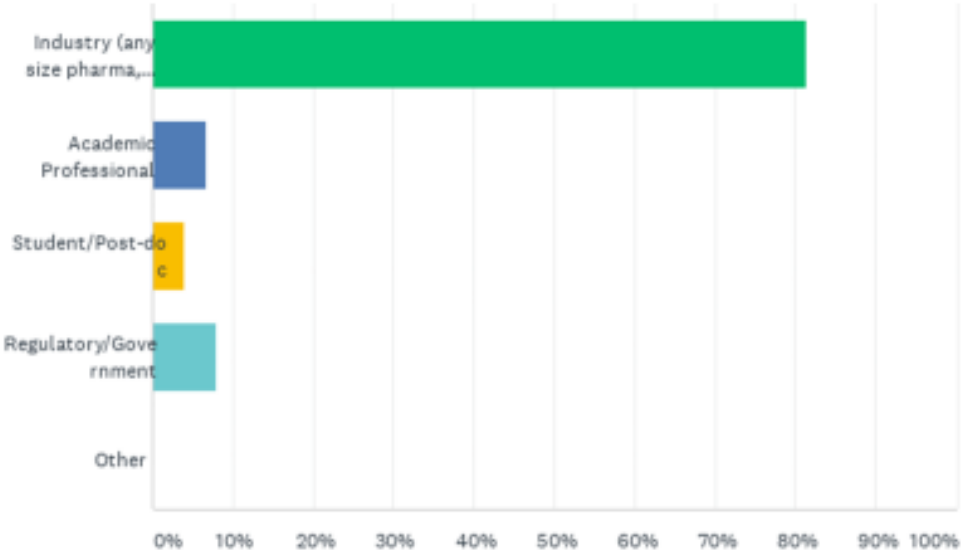
AAPS survey designed by the programming committee preparing for a workshop to discuss protein bioanalysis by mass spec

Programming Committee:

- Brian Booth
- Eric Woolf
- Eric Fluhler
- Faye Vazvaei
- Mark Arnold
- Surinder Kaur
- Wenkui Li

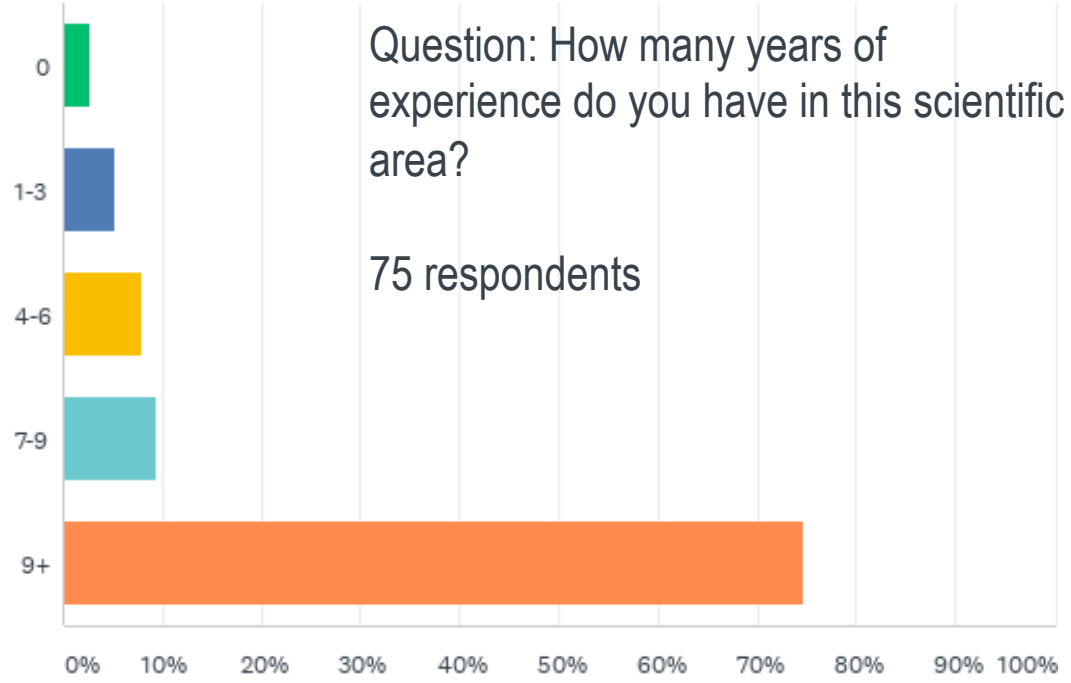
MERCK
INVENTING FOR LIFE

The survey shows that the industry has moved forward…



Primary professional affiliation

75 respondents



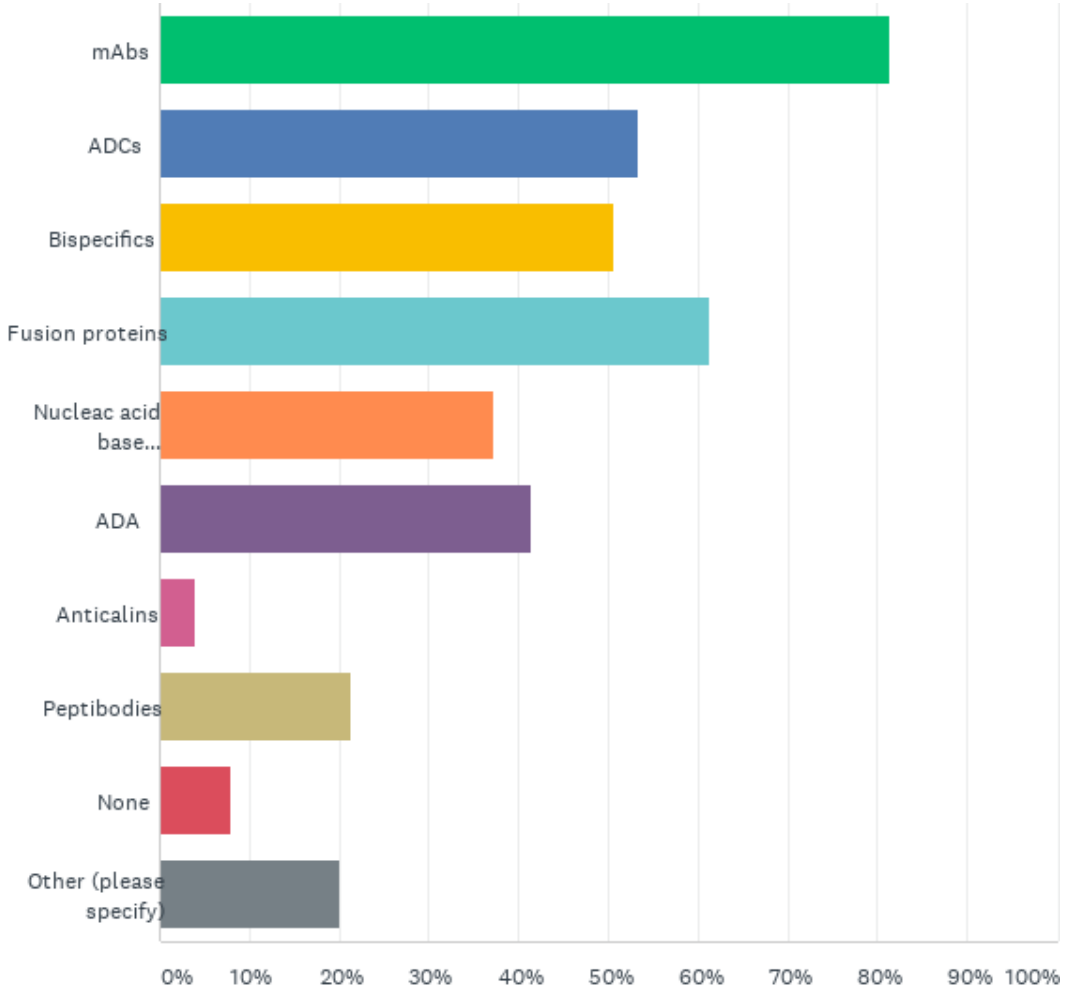
Question: How many years of experience do you have in this scientific area?

75 respondents

Diversity of Modalities

Question: What type of large molecules do you quantify? Select all that apply.

75 respondents



Seminal Whitepapers
Industry consensus papers after > 2 years of discussions

AAPS Protein Bioanalysis by Mass Spectrometry Committee (PBMSC):

Jenkins R, Duggan JX, Aubrey AF *et al.* **Recommendations for validation of LC–MS/MS bioanalytical methods for protein biotherapeutics**. *AAPS J.* 17(1), 1–16 (2015).

IQ Working Group:

Kaur S, Bateman K, Glick J, *et al.* **IQ Consortium Perspective:Complementary LBA & LC-MS in Protein Therapeutics Bioanalysis and Biotransformation Assessment.** *Bioanalysis*, *12* (4), 257– 270 (2020).

Industry Consensus and Recommendations by AAPS PBMSC

Parameter	Protein LBA	Small molecule LC-MS/MS	Protein LC-MS/MS, using a surrogate peptide (recommended by AAPS and IQ)
Calibration curve regression function	Non-linear with 4 or 5 parameter logistic. Anchor points may be used	Linear preferred, non-linear with justification	Linear recommended when possible; non-linear models may be acceptable with some affinity capture methods
Lower limit of quantification (RE, CV)	Within ±25%	Within ±20%	Within ±25%
Calibration standards (RE, CV)	Within 20% (except LLOQ and ULOQ)	Within 15% (except LLOQ)	Within 20% (except LLOQ)
Accuracy and precision (RE, CV)	Within 20% (LLOQ/ULOQ QCs within 25%). Min. 6 runs	Within 15% (LLOQ QC within 20%). Min. 3 runs	Within 20% (LLOQ QC within 25%). Min. 3 runs
Dilutional integrity/linearity	RE, CV within 20%	RE, CV within 15%	RE, CV within 20%
Parallelism	Dilution series CV within 30% using incurred samples	NA	NA; may be used for troubleshooting affinity capture methods

Industry Consensus and Recommendations by AAPS PBMSC

Parameter	Protein LBA	Small molecule LC-MS/MS	Protein LC-MS/MS, using a surrogate peptide (recommended by AAPS and IQ)
Selectivity/specificity			
Non-specific matrix-related interferences: using individual matrix lots, analyzed as blanks and fortified at the LLOQ level. Also evaluate hemolyzed, lipemic, or relevant disease population samples, as appropriate	10 lots; LLOQ: accuracy within 25% for 80% of fortified lots	6 lots; blanks: <20% of LLOQ or <5% of IS. LLOQ: accuracy within 20% for 80% of fortified lots	6–10 lots; blanks: <20% of LLOQ or <5% of IS. LLOQ: accuracy within 25% for 80% of fortified lots
Specific interferences: using LLOQ (and sometimes ULOQ for LBAs) QC samples	Fortified with available material (ADA, soluble target, catabolites) or concomitant drugs (large molecule). Accuracy within 25%	Fortified with available metabolites or concomitant drugs, as appropriate. Accuracy within 20%	Fortified with available material (ADA, soluble target, catabolites) or concomitant drugs, as appropriate. Accuracy within 25%

Additional Considerations by the AAPSJ Whitepaper

Surrogate and monitoring peptides

- No consensus on how many peptides and from which regions to be used for quantification

Use of multiple SRMs

- This is considered to be part of method development and is verified during method validation

Selection of reagents and internal standards (next slide)

Parallelism

- Troubleshooting tool

IQ Seminal Paper

Endorses the AAPSJ recommendations

Discusses the operational considerations

Provides insight on LC–MS & hybrid IA LC–MS strategies

Highlights the need to understand biotransformation

Calls out that there is no regulatory guideline and no consensus on the number of peptides and transitions per peptide

But Where Did These Criteria Come From

Largely empirical

- Based on criteria currently applied to ligand binding and LC-MS assays of small molecules

Do these criteria serve their intended purposes

- For PK we want to make sure that the variability of the assay is below that associated with day to day physiological variability
  - Most biotherapeutics are not administered orally, thus variability attributable to absorption is eliminated
- To ensure that the assay is performing in a consistent manner; thus ensuring that it continues to deliver fit for purpose data
  - Day to day consistency of performance is more important than absolute

Regulatory Input

Booth BP, Furmanski B. Hybrid assays: the next big thing? *Bioanalysis* 10(13), 975–977 (2018).

Regulatory Education for Industry: Regulated Bioanalysis Workshop: Requirements and Expectations--Regulated Bioanalysis of Large Molecules - Bioanalysis 2020, Jinhui Zhang, June 30, 2020.

Regulatory Input, cont'd

Where Does the FDA Stand?

"The FDA has not stated a position on this issue yet due to the lack of data in regulatory submissions, but it has indicated that those criteria would be based on the data generated over time."

SPECIAL FOCUS ISSUE | Protein therapeutics & target quantification by LC–MS

Editorial

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Bioanalysis

Hybrid assays: the next big thing?

Brian P Booth*,[1] & Brian Furmanski[1]

[1]US FDA, CDER, Office of Translational Sciences, Office of Clinical Pharmacology, Silver Spring, MD, USA

*Author for correspondence: Brian.booth@fda.hhs.gov

First draft submitted: 9 May 2018; Accepted for publication: 11 May 2018; Published online: 11 July 2018

Questions from the regulators aimed at assessing and understanding the data

What to Measure?

What peptide should be selected to monitor?

How reliable is the selection of the signature peptide in human studies?

What instrumentation will be utilized in assay validation and during study runs?

How will sample stability be assessed?

Furthermore, what about protein digestion?

What mass fragments should be monitored?

Peptide mass spectra often generate multiple peaks: which ones should be monitored – some representative peaks, or do we need all of them?

Regulatory feedback shared by a sponsor

We have heard of cases where IND and BLA filings were approved using the AAPSJ Criteria.

However,

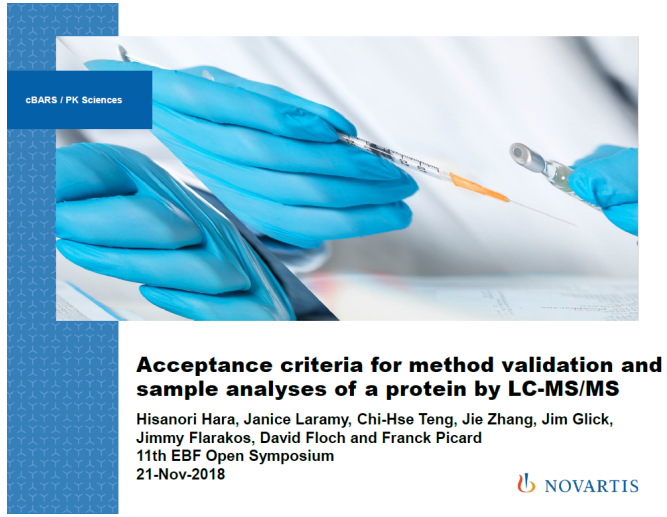
In 2018 we learned from a presentation at EBF

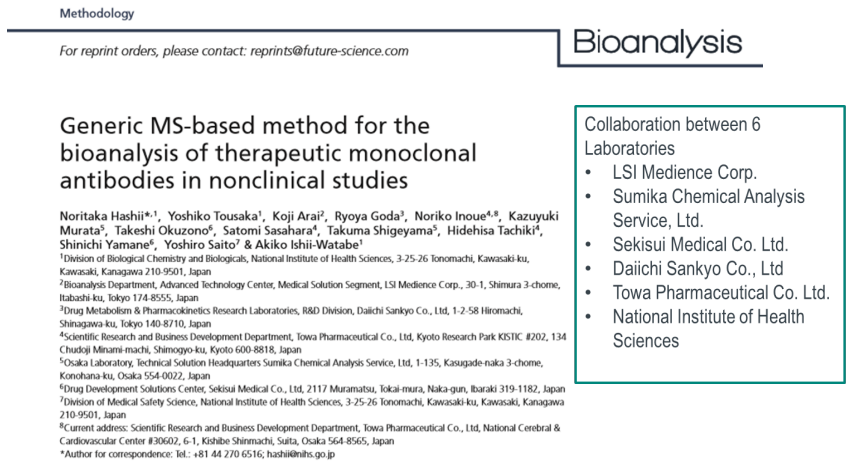
- FDA endorsed the use of LC-MS/MS for the PK assay during development
- The sponsor followed the AAPSJ recommendation for acceptance criteria (20/25%)
- The FDA asked the sponsor to use (15/20%)
- Statistical analysis revealed no relevant difference between the data sets or the PK parameters
- Bioanalytical impact: ~10 additional runs, stability had to be extended, shipment of backup samples

This has further added to a lack of clarity and a need for conversation with the agency

JBF Paper (collaborative activity between NIHS and Industry)– Aim: to develop a simple, robust and low-cost generic LC/MS/MS–SRM approach for targeted IgG1 mAb quantification in nonclinical animal studies…in a collaborative study involving six laboratories, including those from pharmaceutical companies and CROs.

4 out of 6 laboratories met the 15/20% acceptance criteria




Methodology

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Bioanalysis

Generic MS-based method for the bioanalysis of therapeutic monoclonal antibodies in nonclinical studies

Noritaka Hashii*[,1], Yoshiko Tousaka[1], Koji Arai[2], Ryoya Goda[3], Noriko Inoue[4,8], Kazuyuki Murata[5], Takeshi Okuzono[6], Satomi Sasahara[4], Takuma Shigeyama[5], Hidehisa Tachiki[4], Shinichi Yamane[6], Yoshiro Saito[7] & Akiko Ishii-Watabe[1]

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[2]Bioanalysis Department, Advanced Technology Center, Medical Solution Segment, LSI Medience Corp., 30-1, Shimura 3-chome, Itabashi-ku, Tokyo 174-8555, Japan
[3]Drug Metabolism & Pharmacokinetics Research Laboratories, R&D Division, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[4]Scientific Research and Business Development Department, Towa Pharmaceutical Co., Ltd, Kyoto Research Park KISTIC #202, 134 Chudoji Minami-machi, Shimogyo-ku, Kyoto 600-8818, Japan
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[6]Drug Development Solutions Center, Sekisui Medical Co., Ltd, 2117 Muramatsu, Tokai-mura, Naka-gun, Ibaraki 319-1182, Japan
[7]Division of Medical Safety Science, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan
[8]Current address: Scientific Research and Business Development Department, Towa Pharmaceutical Co., Ltd, National Cerebral & Cardiovascular Center #30602, 6-1, Kishibe Shinmachi, Suita, Osaka 564-8565, Japan
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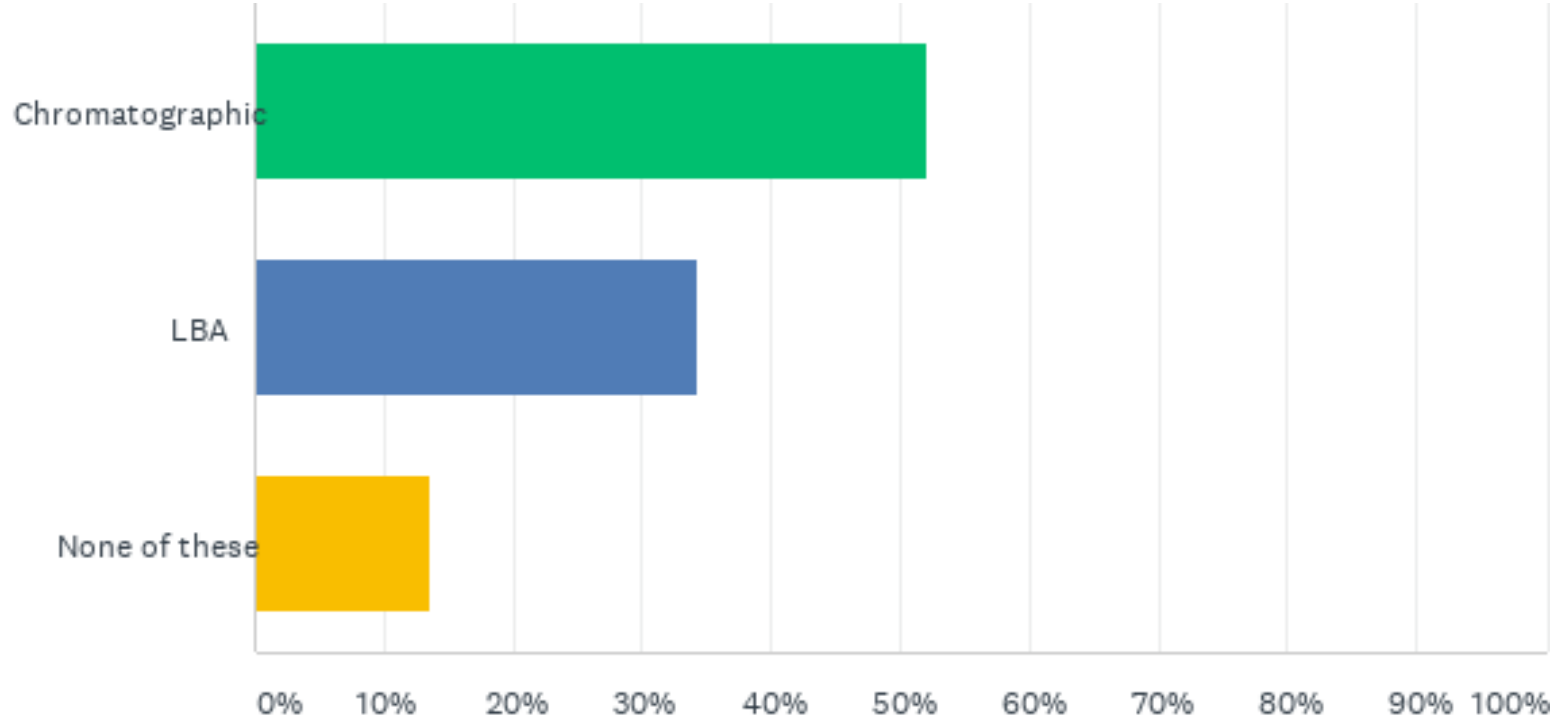
Collaboration between 6 Laboratories

- LSI Medience Corp.
- Sumika Chemical Analysis Service, Ltd.
- Sekisui Medical Co. Ltd.
- Daiichi Sankyo Co., Ltd
- Towa Pharmaceutical Co. Ltd.
- National Institute of Health Sciences

https://www.e-b-f.eu/wp-content/uploads/2018/12/bcn2018-06.-Hisanori-Hara-Novartis-short-version.pdf
https://www.future-science.com/doi/full/10.4155/bio-2019-0253

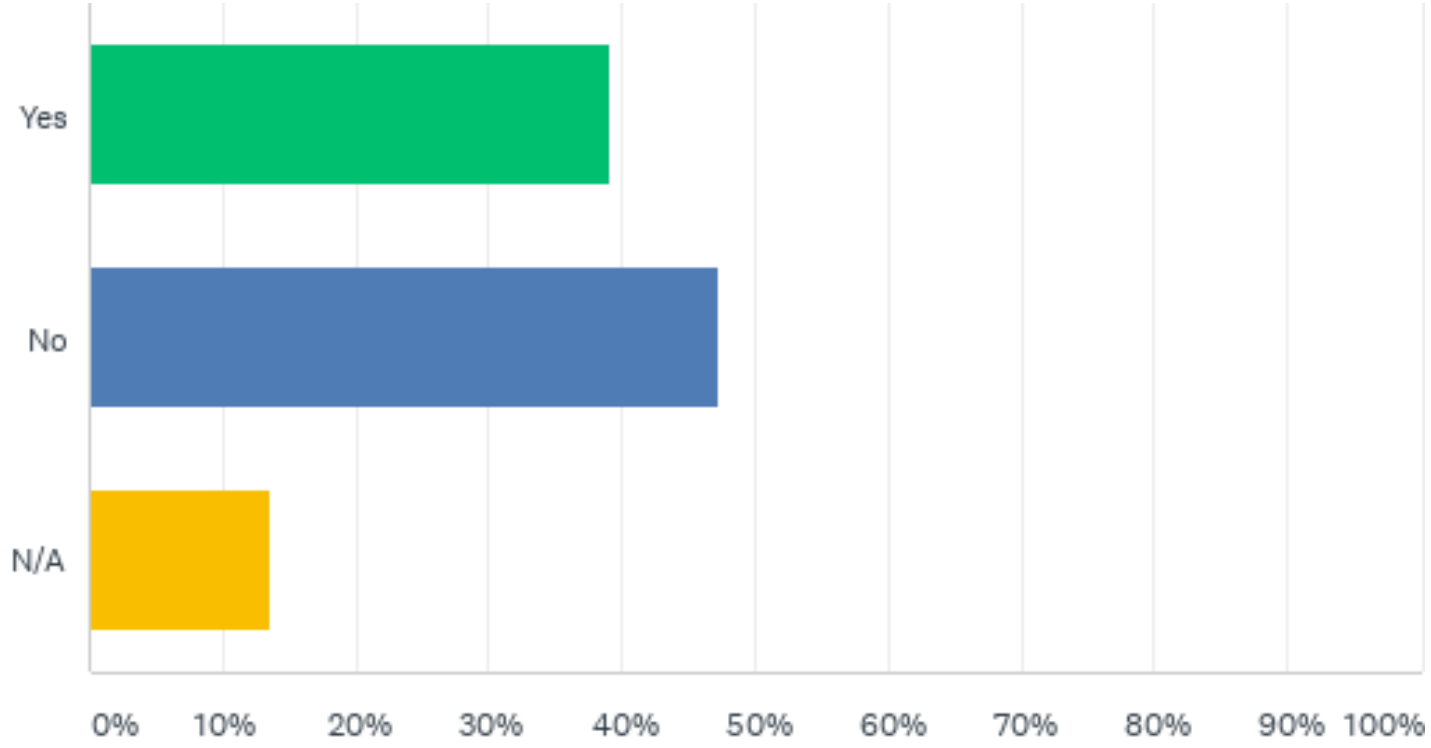
Survey: What acceptance criteria do you use?

Answered: 73 Skipped: 2



Have the data been submitted in support of filings?

Answered: 74 Skipped: 1



It is time….

Time is ripe to have a meeting of minds between the industry and the regulators

- Data driven meeting to share the industry as well as regulatory experience
- Address questions raised by regulatory colleagues
- Reach consensus on the validation requirement and acceptance criteria

We had planned Crystal City 7 on Protein BA by Mass Spec in June 2020

Due to COVID-19 this has been postponed

Stay tuned

Acknowledgments

CC7 programming Committee

- Brian Booth
- Eric Fluhler
- Eric Woolf
- Faye Vazvaei
- Mark Arnold
- Surinder Kaur
- Wenkui Li

All those who contributed to the AAPS survey (AAPS, EBF, JBF, CBF members)

- Joy Davis (AAPS)
- Jinhui Zhang (FDA)
- Dina Goykhman (Merck)
- Brad Roadcap (Merck)
- Hisanori Hara (Novartis)