

Workshop Report ASMS 2013 **Regulated Bioanalysis Interest Group**

Workshop on Challenge in LC-MS/MS Regulated Bioanalysis: Large Molecule by LC-MS Bioanalytical Method Validation (BMV): Status, Challenges, Solutions, Recommendations

Presented by the Regulated Bioanalysis Interest Group (RBIG) at the ASMS 2013 held at the Minneapolis Convention Center, Minneapolis, MN on June 11, 2013

The Regulate Bioanalysis Interest Group has been constituted in 2009 within the ASMS to create a forum for discussion for the numerous ASMS members working in the LC-MS/MS Regulated/GLP Bioanalytical field.

Goal of RBGI

The goal of this interest group is to stimulate discussions on:

- Industry practices in LC-MS/MS regulated bioanalysis
- Interpretation of the latest bioanalytical regulations
- The latest developments, techniques and applications in bioanalysis.

The RBIG actively collaborates with the DMPK interest group on topics of common interest and oral session proposals

The purpose of the annual workshop is to provide an educational forum to discuss issues and applications associated with the LC-MS/MS Bioanalysis.

Multiple sessions were presented during the ASMS 2013 regarding the large molecule analysis by LC-MS

1. MOD am - Biotherapeutics and Biomarkers: Advances in Quantitative Analysis, Ballroom A
2. MOE am - Integrated Qualitative and Quantitative LC-MS for Small Molecule Analysis, Auditorium
3. WOD am - Quantitative Analysis by MS in Drug Discovery and Development: Novel Approaches, Ballroom A.
4. WOD pm - Biomarkers of Drug Response, Efficacy and Toxicity: Novel MS Approaches, Ballroom A

5. ThOC am - Regulated Bioanalysis and Diagnostics using High Resolution LC/MS, Ballroom B
6. ThOD pm - Biomarkers in Drug Discovery and Development, Ballroom A

Supported:

- MOC pm - Clinical Chemistry: Dried Blood Spot Analysis, Ballroom B
- MOE pm - High Mass Accuracy in Drug Discovery and Development, Auditorium

If you want to participate next year on this topic you can

1. Propose topics for ASMS 2014 **Plenary lectures**
2. Propose topics for ASMS 2014 **Oral sessions**
3. Propose topics for ASMS 2014 **Workshop**
4. Participate in the Group **Forum** on ASMS web site

- Submit your proposals to:
 - **fgarofolo@algopharm.com**

Workshop Presentation Program

05:55pm-06:10pm: Challenges and Solutions in Large Molecule Quantification by LC-MS (LM by LCMS): "A Hot Topic in Regulated Bioanalysis"

Dr. Fabio Garofolo (Algorithme Pharma)

06:10pm-06:25pm: Protein Bioanalysis by Mass Spec Activities in North America & Protein LC-MS Method Validation White Paper Consortium –

Ms. Faye Vazvaei (Roche)

06:25pm-07:00pm: Panel Discussion: Recommendations on LM by LCMS from the recent 2012 & 2013 White Papers in Bioanalysis

Expert Panel: **Ms. Faye Vazvaei (Roche); Dr. Jim Shen (Bristol-Myers Squibb); Dr. Gary Schultz (Quintiles)**

LC-MS/MS is increasingly being investigated as an alternative to LBA for evaluating impact of immunogenicity on PK. Refer to the following papers:

- **F. Garofolo et al. – 2011 White Paper in Bioanalysis**
 - **Bioanalysis, September 2011, Vol. 3, No. 18, Pages 2081-2096**
- **B. DeSilva et al. – 2012 White Paper in Bioanalysis**
 - **Bioanalysis, September 2012, Vol. 4, No. 18, Pages 2213-2226**
- **L. Stevenson et al. – 2013 White Paper in Bioanalysis**
 - **Bioanalysis, in progress**

Conclusions: LM by LCMS

- Sensitivity & Selectivity (S&S) is one of the major challenges for LM by LCMS
- LM by LCMS is becoming an emerging analytical technique for analysis of biotherapeutics:
 - Preclinical
 - Immunogenicity
 - Fusion and PEGylated proteins
- HRMS instruments for LM quantification are able to increase the Selectivity while maintaining Sensitivity

Protein Bioanalysis by Mass Spec Activities in North America & Protein LC-MS Method Validation White Paper Consortium

Faye Vazvaei (Roche)

Which guidance should be used for large molecule quantitation by LC-MS? LBA or small molecule, maybe a fit for purpose guidance should be used until a new guidance is written?

Discussions are ongoing within the AAPS Bioanalytical Focus Group Mass Spec Protein Bioanalysis Committee to issue a white paper to cover the following topics:

1. What are the present industry standards in LM by LCMS?
2. Recommendation on cross validation LBA/LCMS for regulatory submissions (LBA orthogonal method)
3. Recommendation of use of LCMS for Immunogenicity (LBA orthogonal method)
4. What to do if LCMS results don't agree with LBA results?
5. How to ensure that trypsin digestion is reproducible in regulate bioanalysis? How do you validate the trypsin digestion?
6. Certificate of Analysis (CoA) for large molecules vs. CoA small molecules. How to address missing info for RSM (e.g.: fusion protein and mAb characterization) and GLP requirements?
7. Criteria to ensure that signature peptide really represent the bio therapeutic? What about protein degeneration?
8. What to do if you had multiple active LMs in circulation but with different Mw? It is not a problem for LBA since they may bind capturing reagent but can you still develop an LC-MS/MS method in this case?
9. What are the criteria for choosing the right IS for LM?

Future activities of AAPS Bioanalytical Focus Group Mass Spec Protein Bioanalysis Committee: perform survey and form small group to discuss about targeted area of interest such as analysis using micro flow, HRMS, sample preparation etc.

Panel Discussion

Question: Suggestions /questions related to different approach for sample preparation:

Approach #3 preferred (isolate the protein and digest), how do you account for the yield of recovery for affinity

Answer: We assume that an excess of reagent is added. This technique is not really an extraction, but mostly an enrichment technique.

What would the agencies say about not having a yield evaluated; this could be questioned by agencies? The use of an IS is discussed in the industry.

Make sure you demonstrate your point of equilibrium and do a time titration and ensure you reach a steady state of binding.

As nobody represents the regulatory agencies, no one can confirm for sure that it will be accepted.

Question: Since Approach #2 and #3 include an immunoaffinity capture step it is similar to developing an ELISA method, isn't it the same as developing a LBA? Then which approach should be used?

Answer: No, non specific capture Ab is required.

Comment: Good ELISA needs two Ab, where as this only needs one.

Question: Is there any example for regulated analysis of a LM by LC-HR MSMS accepted by agencies?

Answer: We do not know whether the data was submitted by the sponsor, although analysis was performed. FDA back up your data with good science

Question: How about validation of the instruments?

Answer: API 5600 is CFR part 11compliant and our validation is ongoing.

If the method developed doesn't follow the available guidelines, a good scientific explanation and validation plan ahead of time is needed.

Question: Can you give examples of which kind of tests would not meet the acceptance criteria of the small molecule guidance, because it seems that this guideline should be used?

Answer: No SIL IS could be available, therefore the variability cannot be capture and can be above small molecule criteria's. Also a digestion might not be tracked depending on the IS used.

If permitted use small molecule criteria

Question: EMA has a white paper and different organizations are trying to interpret the guidelines differently can this be use by regulatory bodies against the industry?

Answer: Should the interpretation of the guidelines should be performed through good science

White papers are a snapshot in time and it's a work in progress, sharing ideas and experiences