Non-standard technique in a regulated environment: What considerations come with it?

By Connor Walker (Arcinova)

Defining non-standard techniques

- Or better yet, what do we mean by standard techniques?
  - Defined as an agreed upon technique as the ideal way to get a desired result
  - Examples include chromatographic assays such as LC and GC

- In contrast non-standard techniques are:
  - More niche in application
  - Not widely adopted in the given field
  - Typically excel in their respective area of application

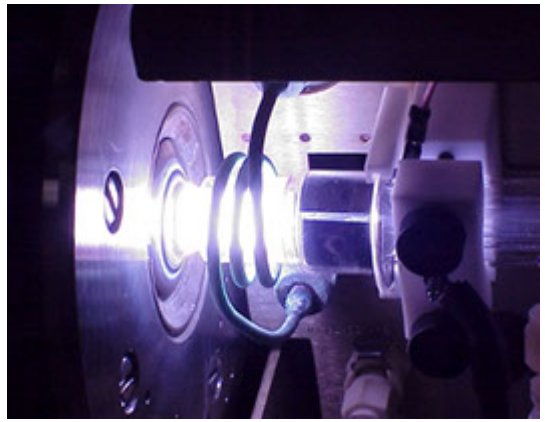
Why choose non-standard?

- The reasoning
  - Typically non-standard techniques are chosen on the basis that they are the best way to measure the analyte e.g. trace elements
- Trace elements
  - Accurate and reliable trace element data can be critical to the success of drug trials
  - While there are alternative methods, ICP-MS is most sensitive and efficient
- For this reason, bioanalytical labs such as Arcinova may use ICP-MS for bioanalysis

What is ICP-MS?

- Inductively coupled plasma mass spectrometry

- Mode of action
  - Designed for elemental analysis (excels at trace element analysis) utilising a argon plasma torch to atomise and ionise samples



- Robust, sensitive, and rapid method of analysis
- Niche in application and by nature a destructive method
- <u>No specific regulatory guidance for application in regulated work</u>

The guidelines

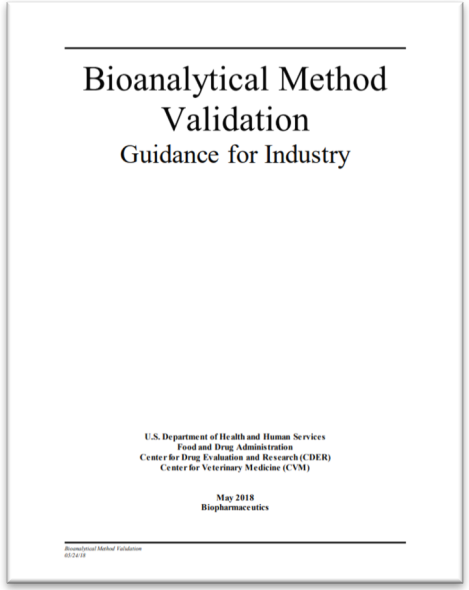
- *Bioanalytical method validation: Guidance for industry (2018)* - FDA

- Similar guidance published by the EMA with minor differences

- Harmonization of regulatory documents from different territories in progress (ICH M10)

- Lists recommended tests to ensure bioanalytical methods produce reliable and reproducible data

- Critical for GLP studies as data is essential for the understanding new drug molecule interactions in the body


Bioanalytical Method Validation
Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

May 2018
Biopharmaceutics

Interpreting the guidelines

- Current regulatory guidelines specify tests for Chromatographic (CC) and Ligand Binding Assays (LBA), not ICP-MS

  - These tests should be perceived as trying to understand aspects that are critical for a robust assay, rather than just checkboxes for CC and LB assays.

- From *The 2018 FDA BMV Final Guidance* presentation by the FDA, four fundamental questions are being investigated in the guidelines:

  - Does the method measure the intended analyte(s)?
  - What is the range of measurements that provide reliable data?
  - What is the variability in these measurements?
  - How does sample collection, handling, and storage affect the reliability of the data?

Applying the guidelines

- Most CC based assay tests from the guidance can be applied
- CC tests cover most concerns, however they do not take into consideration the unique factors that arise from ICP-MS assays
- Directly applying just CC validation tests can lead to ‘gaps’ in compliance and assay reliability.
- The ‘questions’ we are being asked are not answered in a satisfactory manner
- Answering these ‘questions’ should be approached in a scientific manner, taking into consideration unique technique related factors to ensure assay reliability both in terms of regulation and performance

So what are these considerations?

Sample preparation / storage

- Sample digestion during preparation
  - Ensure the release of bound element components
  - ICP-MS standard nebulisers can typically only handle up to 2% solid matter

- Sample storage
  - Sample storage should mimic the container used in sample collection
  - Elements (even different forms of the same element) can have varying volatility, precipitation, and container adsorption properties

- Internal standard
  - Elemental rather than physiochemical
  - IS should have ionization/atomization behaviour similar to the analyte

Analyte specificity

- Analyte(s) may be endogenous
  - Can be found in the environment, plastics, reagents, matrix etc.
  - Examples include Copper & Iron
  - Contamination results in unexpected spikes in analyte concentration in samples, undermines run data

- Sensitive assays with endogenous analytes should have contamination control, taking the following into account;
  - Materials and technique employed used for sample processing
  - Proper sample homogenization
  - Observation and investigation of abnormally high results

Analyte interference

- Elemental analysis is susceptible to 3 types of interference:
- Isobaric
  - Result of equal mass isotopes from different elements in solution (e.g. ^{58}Fe and ^{58}Ni overlap)
- Double charged
  - Double charged element isotopes that contain twice the mass of the analyte (e.g. $^{206}Pb^{++}$ (m/e = 103) equivalent to ^{103}Rh)
- Polyatomic
  - Combination of two or more isotopes from different elements (e.g. ^{56}Fe interfered by $^{40}Ar^{16}O^{+}$)
- Can be mitigated via reaction/collision cell and careful selection of isotope(s) that will be analysed.
  - Such interferences should be noted during validation as well as ways in which to mitigate them

Machine parameters

- ICP-MS has both tuneable and non-tuneable parameters
- Tuneable factors include;
  - Torch position, nebuliser flow, carrier/reaction gas and ion Optic lens voltage
  - ICP-MS capable of performing auto-tune on use by use basis for optimisation
  - Validation of these tuneable features **not** required

- Non-tuneable factors include;
  - Sample pump tubing size and uptake rate, spray chamber type and temperature, interference cones etc.
  - Validation of these parameters **is** required, and should be maintained definitively

System suitability

- Consideration to the ICP's system suitability is important during validation as it can potentially play a role in the sensitivity of an assay
  - Some elements tend to be 'sticky' and cause a memory effect, resulting in increased background when analysing samples
  - Conversely some elements/matrices require pre-conditioning

- System suitability also confirms the non-tuneable setup on the machine is correct.

- It is also good practice to check the machine's suitability before committing samples, especially if they only have the volume for one injection

Answering the questions 1

- By combining both recommended CC tests and the considerations of ICP-MS we are able to answer the ‘questions’ posed

- Does the method measure the intended analyte(s)?
  - Selectivity, sensitivity etc. tests demonstrate this
  - Consideration to choice of isotope(s), analyte specificity and interference ensures intended analyte(s) are measured

- What is the range of the measurements that provide reliable data?
  - A&Ps and dilution integrity tests prove reliability of the selected range
  - Contamination control and system suitability ensure low background for lower ranges

Answering the questions 2

- What is the variability in these measurements?
  - Haemolysis, matrix selectivity and factor etc. tests help assess variability
  - Accounting for isotope choice, consistency in machine variables, choice of IS, and system suitability
  - Contamination control to mitigate spontaneous variability in endogenous assays

- How does sample collection, handling, and storage affect the reliability of the data?
  - Long term, short term, and freeze / thaw stability tests confirm reliability
  - Characteristics of elemental volatility, precipitation, and adsorption are taken into consideration

- Possible gaps in compliance are mitigated or removed entirely demonstrating the integrity of the assays developed

Discussion

- Validating non-standard bioanalytical methods can be straight-forward as the fundamental questions that need to be answered are the same as those outlined in the guidance documents, but consideration should be given to variables associated with the technique to ensure full compliance

- Scientific judgement to ascertain what tests are needed, which are not, and what other factors (unique to the technique) should be taken into consideration to ensure regulatory compliance

- When validating non-standard techniques for regulatory work, the technique should not be forced to fit the guidance. Instead asking, how can the guidance fit the technique, and does it do so in a compliant manner

THANK YOU

References

- Food and Drug Administration. (2018). *Bioanalytical Method Validation Guidance for Industry*. Available: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Last accessed 24th Jun 2020.
- European Medicines Agency. (2011). *Guideline on bioanalytical method validation*. Available: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Last accessed 19th Jun 2020.
- Levine K.E., Tudan C., Grohse P.M., Weber F.X., Levine M.A., Kim, Y.S.J.. (2011). Aspects of bioanalytical method validation for the quantitive determination of trace elements. *Future Science*. 3 (15), 1699-1712.
- Weiling J., Meijering H., Heuveln, F.. (2012). Inductively coupled plasma-MS in drug development: bioanalytical aspects and applications. *Bioanalysis*. 4 (15), 1933-1965.
- Cubadda, F.. (2007). Chapter 19 - Inductively coupled plasma mass spectrometry. In: Yolanda, P. *Food Toxicants Analysis*. Spain: Elsevier Science. 697-751.
- Heuveln, F., Meijering, H., Wieling, JP. (2012). Inductively coupled plasma-MS in drug development: bioanalytical aspects and applications. *Bioanalysis*. 4 (15), 1933-1965.
- Booth, B., FDA. (2019). The 2018 FDA BMV Final Guidance. In: WRIB *13th WRIB; M2 Training session; Implementation of 2018 FDA BMV Final Guidance*. USA: WRIB. 5-14.
- Biss, J. Whitmire, M.L., Osredkar, A., Ammerman, J., Marshall T., Huang, C., Lisio, P.D., Ehringer, K.. (2011). Full Validation of a High Resolution ICP-MS Bioanalysis Method for Iron in Human Plasma with K2EDTA. *Journal of Chromatography & Separation Techniques*. 4 (8), 1-7.

References

- Images
- 1) 5977B GC/MSD, GC/MS instruments, Agilent, website: https://www.agilent.com/en/product/gas-chromatography-mass-spectrometry-gc-ms/gc-ms-instruments/5977b-gc-msd. Accessed 17th June 2020
- 2) Billy Joe Molloy, 2015, ACQUITY UPLC System with Xevo TQ-S Mass Spectrometer., figure 1. Accessed 20th June 2020
- 2) Alain Desprez, 2016, Agilent solutions for Nanoparticles analysis ICP-MS, ICPMS-MS, software. Last accessed 20th June 2020
- 3) Elemental analysis, 2016, Inductively coupled plasma, website: https://www.elementalanalysis.com/icp.html. Accessed 20th June 2020
- 4) Guidance for industry – Bioanalytical method validation, 2018, website: https://www.yumpu.com/en/document/view/21736050/guidance-for-industry-bioanalytical-method-validation . Accessed 22nd June 2020
- 5) ICP-MS cones, 2020, Analytical west, website: https://www.analyticalwest.com/products/icp-ms-accessories/icpms-cones.html. Accessed 21st June 2020
- 6) ICP-MS tubing, 2019, ICP-MS resource hub. Website: https://www.agilent.com/en/promotions/icp-ms-resource. Accessed 21st June 2020