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Applied Translational Medicine

THE STATISTICAL BENEFIT OF PERFORMING GLP BIOANALYSIS USING ASSAYS THAT HAVE REDUCED VARIABILITY

Raymond H. Farmen, Ph.D.
Vice President, Global Bioanalytical Services

Kirk Newland, Don Graff and Michelle Combs, Ph.D.

Presentation Outline

- Definition of Modified Risk Tobacco Product (MRTP)
- Challenges to develop MRTPs
- Biomarkers as a tool to support MRTP Applications
- Describe the key attributes for a fit-for-purpose Bioanalytical Tobacco Assay (BTA)
- Demonstrate how well designed Bioanalytical Tobacco Assays can improve statistical power and reduce the cost of MRTP development
- Summary
- If time permits - compliance

Modified Risk Tobacco Product (MRTP)

- MRTP means “any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products.”
- MRTPs can be introduced using 1 of 2 paths:
  - Path # 1: “risk modification order”
  - Path # 2: “exposure modification order”

Source: FDA Draft Guidance: Modified Risk Tobacco Product Applications

Modified Risk Tobacco Product (MRTP)

- **Path # 1** - Risk Modification Order (Section 911(g)(1)) requires
  - Evidence that MRTP significantly reduces harm and the risk of tobacco related diseases to individual tobacco users
  - Benefit the health of the population as a whole
- **Path # 2** - Exposure Modification Order (Section 911(g)(2)) requires
  - Scientific evidence of substantial overall reductions in exposure to the harmful substance(s)
  - The available scientific evidence demonstrates that a measurable and substantial reduction in morbidity/mortality among individual users is reasonably likely in subsequent studies
  - The order would be appropriate to promote the public health

Source: FDA Draft Guidance: Modified Risk Tobacco Product Applications

Challenges to Establishing Reduced Harm Path # 1: Risk Modification Order

- Epidemiological studies are the “gold standard”
- Epidemiological studies require;
  - Long times due to long latency period of smoking related diseases such as cardiovascular diseases, COPD, lung cancer etc. to observe clinical end point
  - Large number of participants with well-matched confounding factors such as age, sex, ethnicity, and other lifestyle factors
  - Subject compliance to the study protocol over long period of time
  - Low subject attrition rate

Challenges to Establishing Reduced Harm Path # 2: Exposure Modification Order

- MRTP development requires faster and more controllable methods to assess smoking related diseases other than epidemiological studies
- Biomarkers offer an alternative and cost-effective approach for evaluation of potential harm reduction from MRTPs during product development
- The profiles of biomarkers may be used to understand biological events from smoke inhalation to disease manifestation
- Endpoint is the biological effect in response to smoking as opposed to disease manifestation

Biomarker Definition

- 'Biomarker' is a good example of a term whose dictionary definition is not keeping pace with the word's changing significance in the real world

- The US National Institutes of Health definition:
  - "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention"

Examples of Tobacco Biomarkers

- Biomarkers of exposure:
  - Nicotine in plasma
  - Nicotine equivalents in urine
  - Polycyclic Aromatic Hydrocarbons in urine
  - Tobacco Specific Nitrosamines in urine
  - Aromatic Amines in urine
  - Mercapturic Acids in urine

- Biomarkers of effect:
  - 11-dehydro-thromboxane B2
  - Isoprostanes like 8-iso-PGF2 (type III)

"Fit-for-Purpose" Method Validation of Biomarkers to Support MRTP

- Types of biomarkers based on the intended purpose
  - Exploratory
  - Supportive
  - Definitive

"Biomarker validation processes are continuous and iterative, and driven by the intended purpose of the biomarker data"

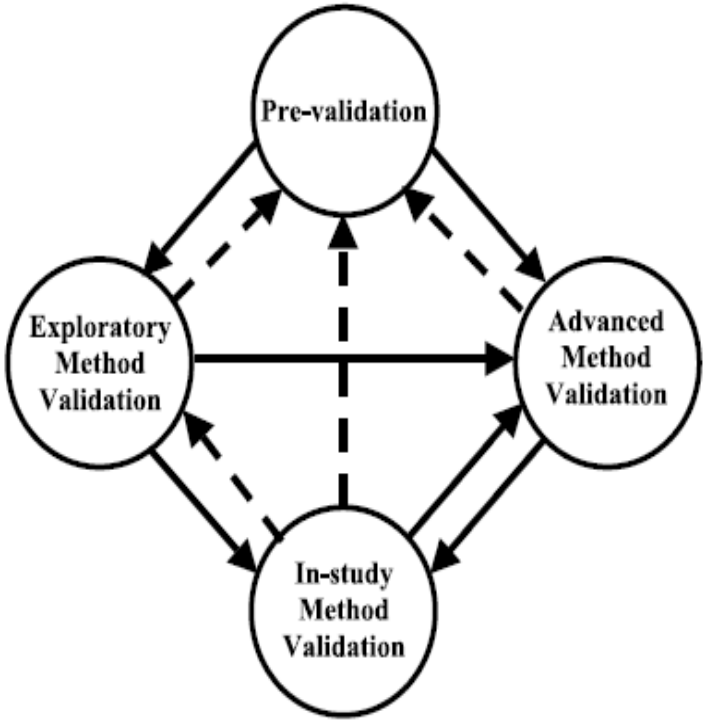

Fig: Conceptual diagram of fit-for-purpose method validation.

Source: Lee et. al (2006)

Where do Bioanalytical Tobacco Assays for biomarkers fit in this validation process?

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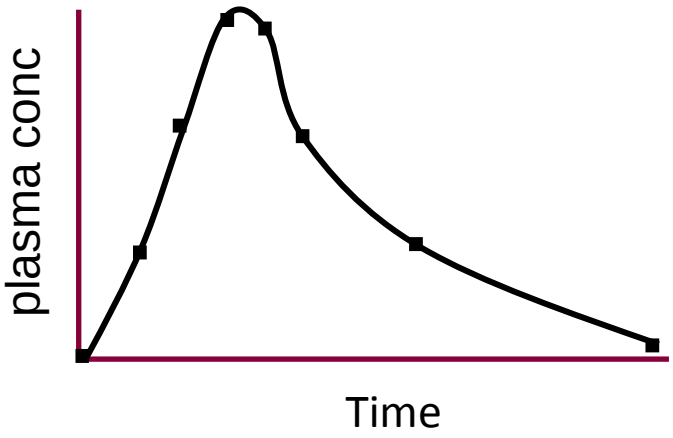
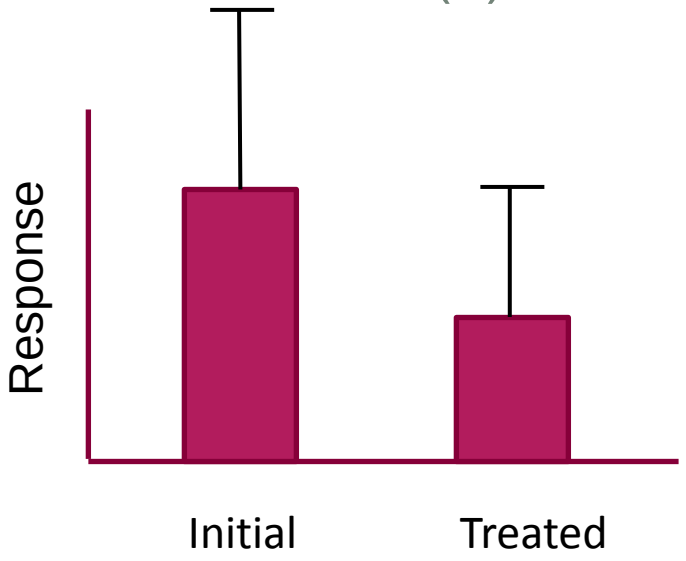
Biomarkers to Support MRTP Fit-for-Purpose

LAW: Exposure modification order (Section 911(g)(2)) requires “scientific evidence of substantial overall reductions in exposure to the harmful substance(s)”



Bioanalytical PK assay



Bioanalytical Tobacco Assay

Is a bioanalytical PK assay the same as a Bioanalytical Tobacco Assay if the purpose is different?

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Method Validation Guideline for PK Assays:

- Bioanalytical Guidance – assay must be:
  - Selective ✓
  - Sensitive ✓
  - Accurate
  - Precise ✓
  - Stable
    - Sample collection and handling
    - Freezer (-20°C or -80°C)
    - Freeze/Thaw
    - UV light sensitivity
    - Bench-top
    - Pre-extraction
    - Post-extraction
  - DOCUMENTED !

Focus on how these parameters differ between a bioanalytical PK assay and a Bioanalytical Tobacco Assay

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Selectivity - Contamination

- Bioanalytical PK assay - In a PK assay you don't have to worry about the drug that you're measuring being present anywhere else other than the sample.
- Bioanalytical Tobacco Assay – contamination of the sample from other sources is a concern:
  - Nicotine is ubiquitous!
    - Lower Limit of Quantification (LLOQ) = 0.2 ng/ml
    - Nicotine contamination in transfer tubes > 4 ng/ml
    - Methanol rinsed tubes, pipettes, etc. eliminates contamination
    - Nicotine free lab
  - O-toluidine, an aromatic amine, was shown to leach from certain urine collection containers (4X > LLOQ) and other types of plastic ware.

Selectivity – Concomitant medications

- Acceptance Criteria: The response in individual blank matrices should be less than 20% of the LLOQ for the analyte in at least 80% of the tested individual matrices
- Bioanalytical PK Assay – Required to show that con-meds do not interfere with drug, metabolites or internal standard. Celerion tests an OTC cocktail when evaluating assay selectivity.
- Bioanalytical Tobacco Assay – What do you test?
  - Example - for NNN we tested all other TSNAs and their precursors. Had to work around nor-nicotine interference.
    - LLOQ for NNN = 0.20 pg/ml
    - Nor-nicotine = ng/ml

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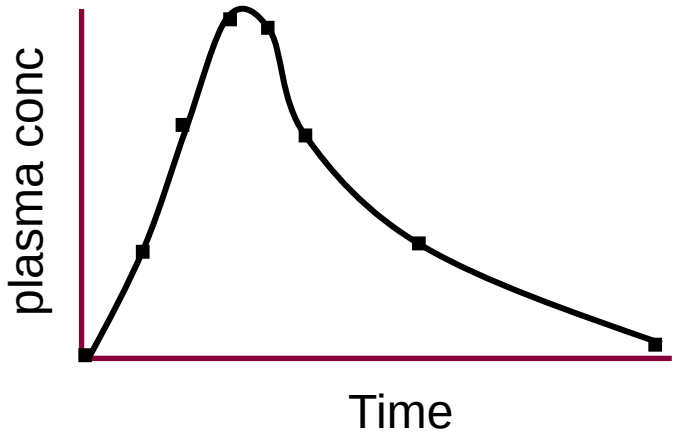
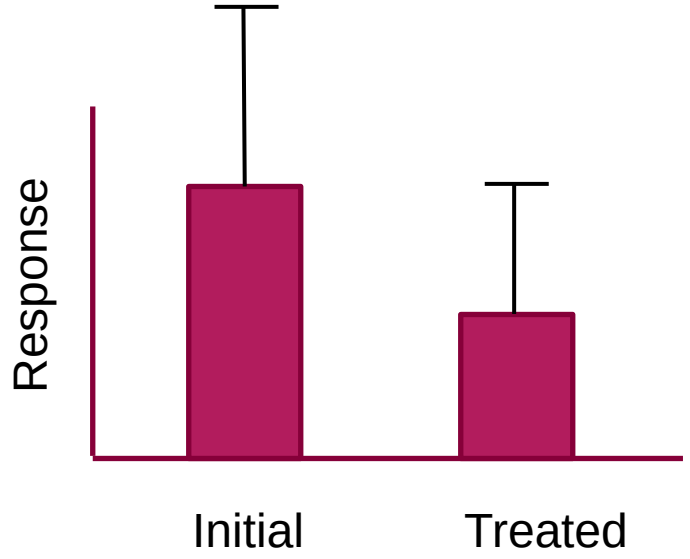
Sensitivity

- Sensitivity (Lower Limit of Quantitation - LLOQ):
  - LLOQ is the lowest concentration of analyte in a sample which can be quantified reliably with acceptable accuracy and precision
  - The analyte signal of the LLOQ sample should be at least 5 times > the signal observed in blank samples



Bioanalytical PK assay



Bioanalytical Tobacco Assay

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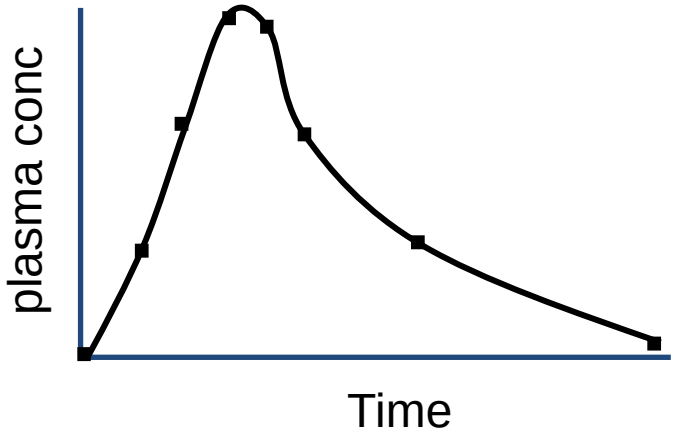
Sensitivity

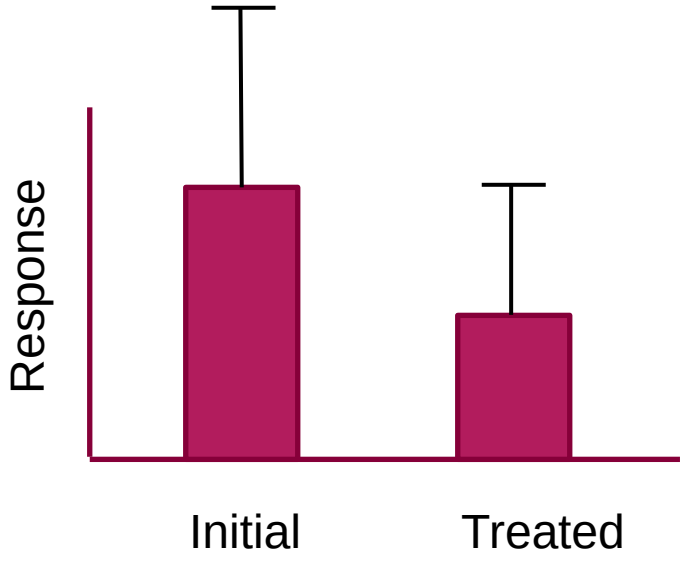
- Bioanalytical PK assay – A sample < LLOQ is not reported and has minimal impact upon PK analysis.
- Bioanalytical Tobacco Assay – Several samples < LLOQ can kill your power when you're trying to show that two products are statistically different.
  - Celerion has one of the most sensitive assays for the measuring NNN with an LLOQ = 0.70 pg/ml
  - It was found that >20% of the clinical samples for a MRTP were below LLOQ. It is difficult (if not impossible) to do comparative statistics when a lot of the results are <LLOQ
  - Solution: new method was developed using state-of-the-art LCMSMS technology with an LLOQ = 0.20 pg/ml.

Precision

- The precision of the analytical method describes the closeness of the repeated individual measures of an analyte
- Bioanalytical PK Assay Acceptance criteria: The coefficient of variations (CV) for with-in run imprecision and between-run imprecision should not exceed 15% (20% at LLOQ)
- Bioanalytical Tobacco Assay - Is the PK assay acceptance criteria acceptable?

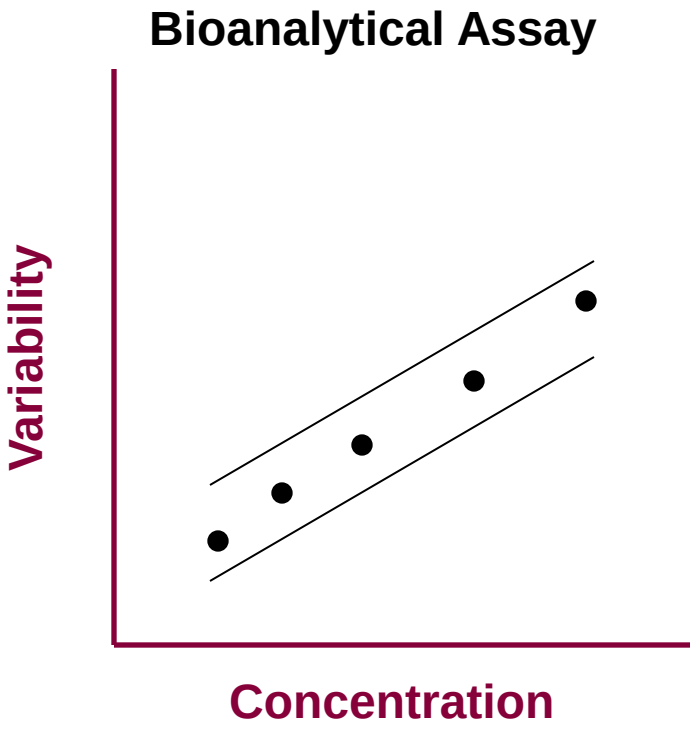


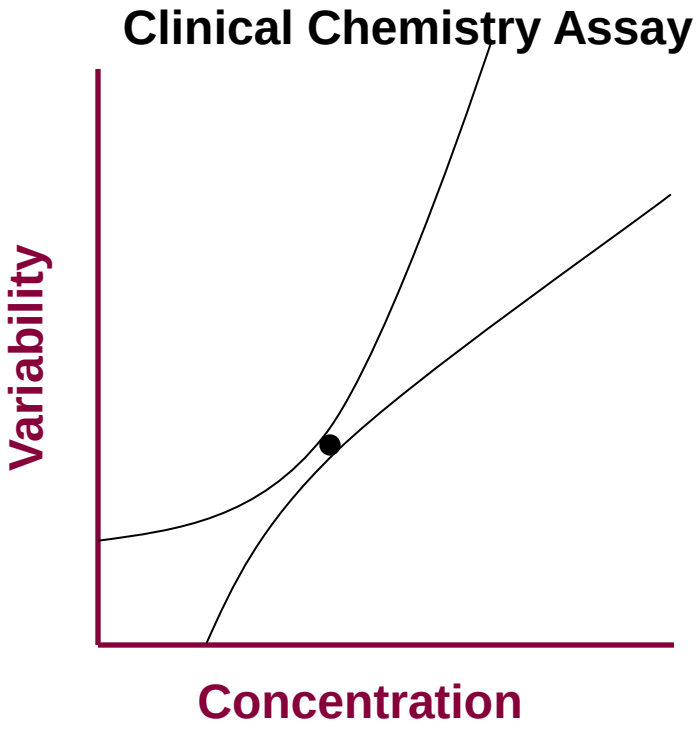
Bioanalytical PK assay



Bioanalytical Tobacco Assay

Precision - Assay variability comparison





The design of bioanalytical assays using multiple standards produces constant variability over a large concentration range.

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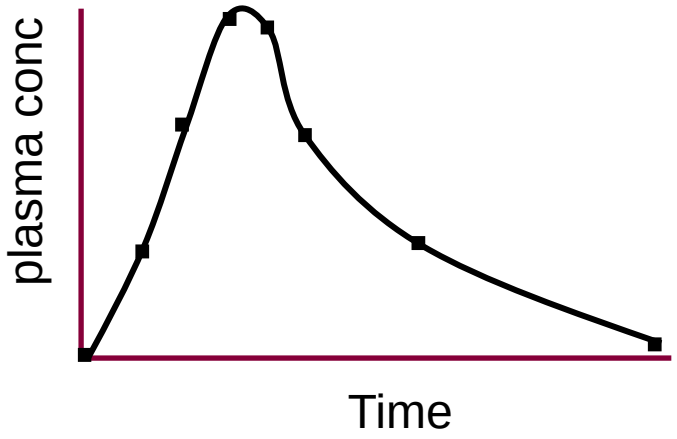
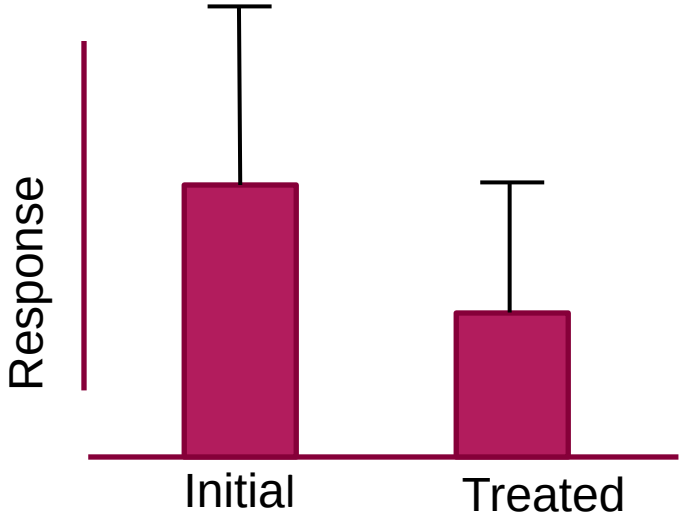
Precision - Focus on Assay Variability of Bioanalytical Tobacco Assays



For PK needs ± 15% precision is OK



For stats ± 15% precision may not be OK

- Emphasis on reducing assay variability for assays used for statistical comparison (most Celerion tobacco assays have variability < 10%).
- Can reduced assay variability result in fewer participants being dosed?

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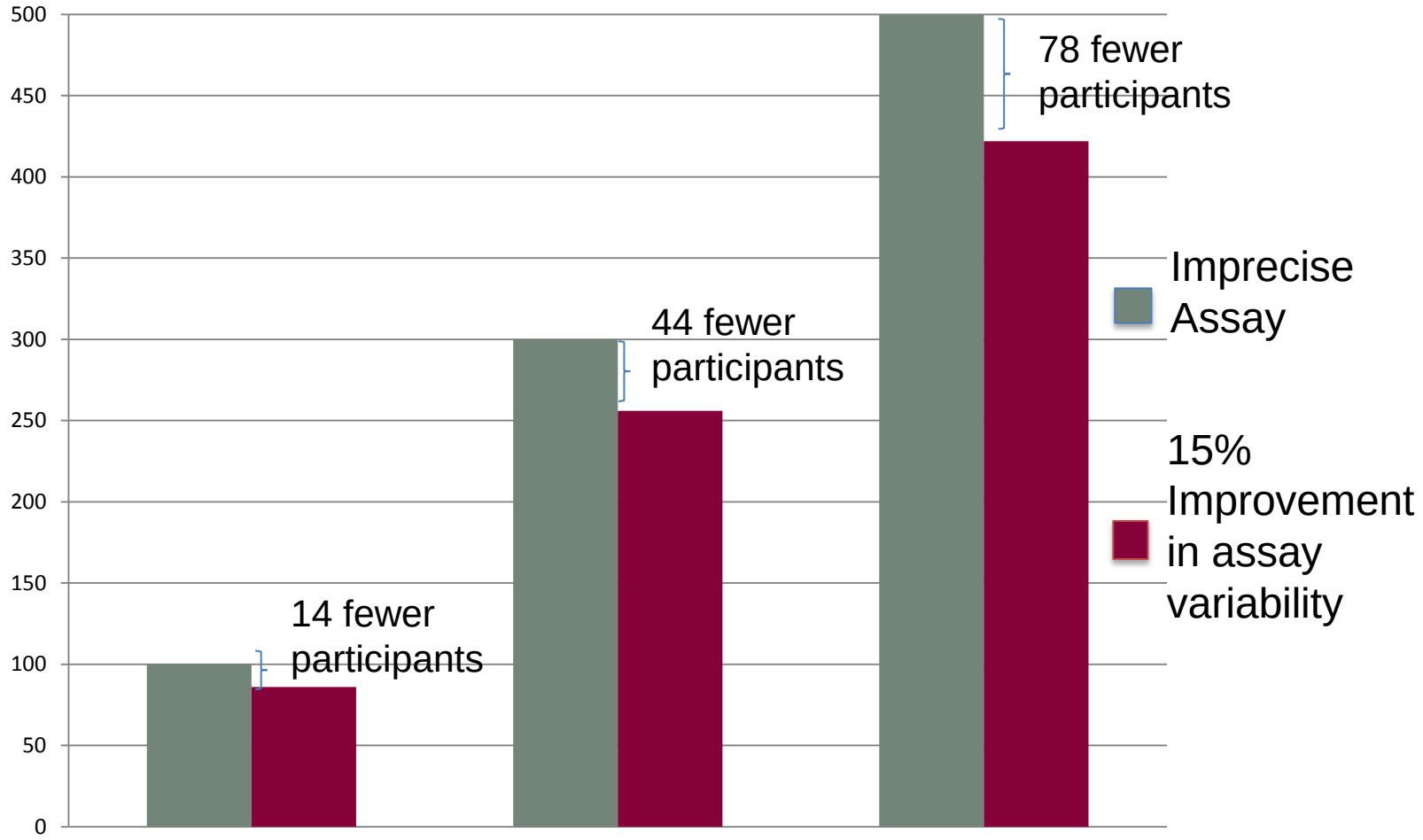
Precision - Assay variability's impact on number of participants



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Summary

- Biomarker analysis provides a regulatory path to generate "scientific evidence of substantial overall reductions in exposure to the harmful substance(s)"
- Choosing appropriate panel of biomarkers is critical to the success of MRTP applications
- Choosing appropriate assays to measure biomarkers is also critical to the success of MRTP applications
- Fit-for-Purpose Method Validation: a bioanalytical PK assay ≠ a Bioanalytical Tobacco Assay ≠ a clinical chemistry assay
- Bioanalytical Tobacco Assay: selectivity, sensitivity and precision are of greater importance and are usually harder to achieve when compared to other assay types.
- Bioanalytical Tobacco Assays have the ability to streamline MRTP development and reduce its cost by achieving statistical power with fewer participants!

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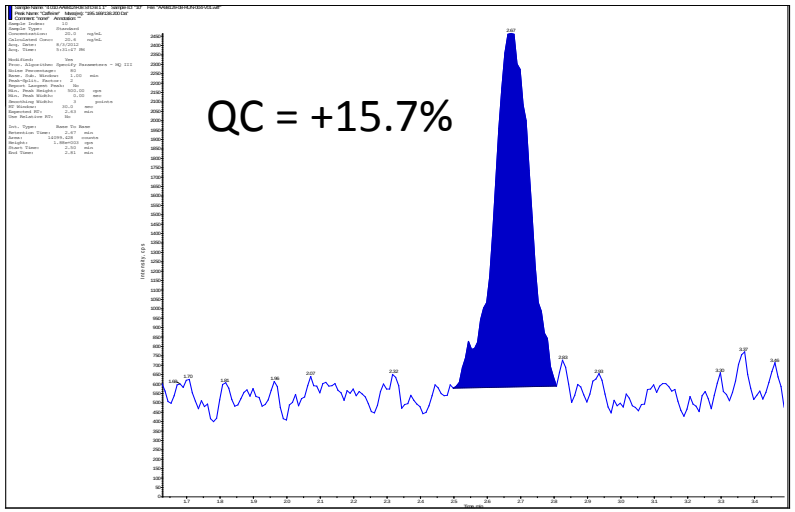
Compliance

- Bioanalysis is all about compliance! (How the EBF meeting opens each year!)
- Regulated bioanalysis must follow GLP and GCP guidelines (GCP in recent EMA guidance)

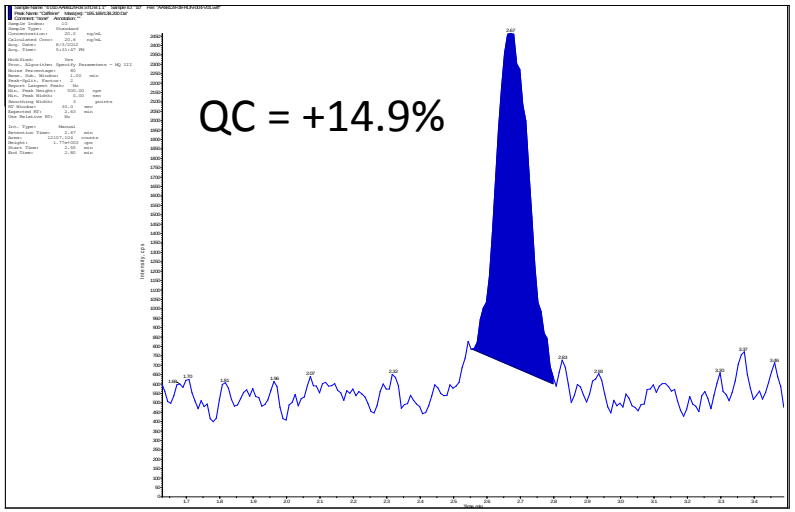
- GLP basics
  - Say what you're going to do
    - SOPs
    - Bioanalytical Study Plan
    - Validated Bioanalytical Method
  - Do what you said you were going to do
  - Document it!

Documentation – Chromatographic Integration

- Big Deal – the vast majority of serious audit findings involve scientists modifying integration parameters for QCs to get them to pass acceptance criteria (± 15%)



Consistent with other samples
Original computer generated



Inconsistent with other samples
Manually drawn

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Documentation – Chromatographic Integration

- Big Deal – A significant number of serious audit findings involve scientists modifying integration parameters for QCs to get them to pass acceptance criteria.
- Best Practice – Review all chromatographic integration and manually redraw baselines then “lock-it-down” prior to performing regression analysis. It should be very difficult to modify integration following regression analysis.
- All changes to any data must be documented:
  - Who is making the change
  - When was the change made
  - Why
- Advantages of an Electronic Laboratory Notebook!

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