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Update on ANVISA Bioanalytical Method Validation (BMV) Guideline

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Brazilian BMV

- This presentation is intended to show the main aspects of the ongoing revision of the Brazilian guideline on BMV
  - The draft called CP33 has been published in 28/June/2011 for contributions
  - Deadline: 28/August/2011 (closed)
- Brazilian BMV
  - Focused on relative bioavailability / bioequivalence studies
  - Last issue: 2003
  - The revised version is intended to be more comprehensive
    - It will be the first guideline on BMV in Latin America
- The Coordination of Bioequivalence (COBIO) of ANVISA (Brazilian Health Surveillance Agency) is leading the revision process
  - ACBIO through its department (focus group) of Bioanalysis (ACBIO-Bioanalises) has made meetings with COBIO/ANVISA on this issue

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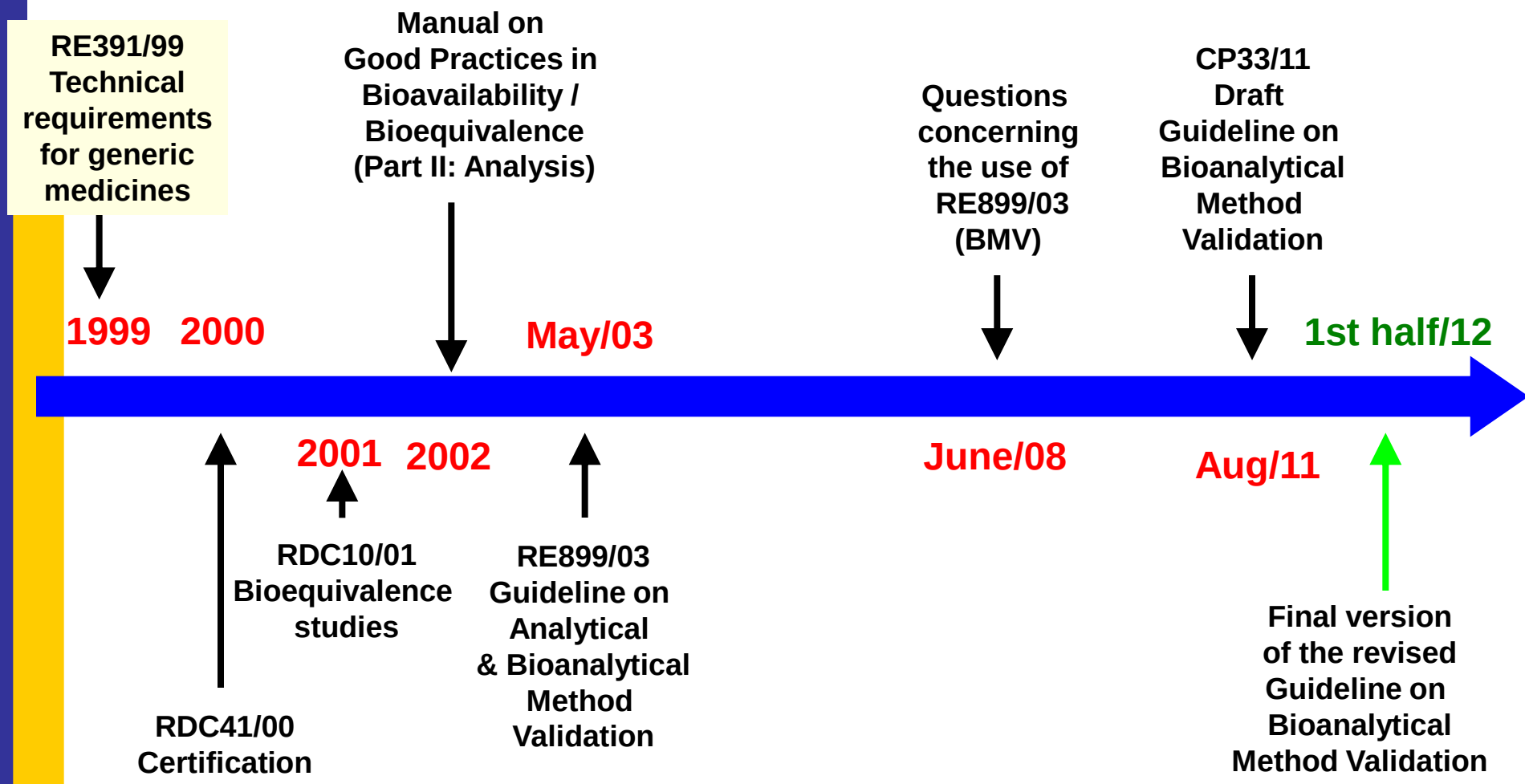
Historical aspects on Brazilian BMV



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Brazilian Guideline on BMV

- RE899/03
  - Glossary: few definitions
  - Written mostly for LC method
    - LBAs not considered
    - Endogenous compounds also not considered
  - Reanalysis not covered
  - Application to the routine analysis
  - Not discussed with bioanalytical community
    - Draft not published

- CP33
  - Glossary: definitions improved
  - Other analytical techniques (non-chromatograpic) could be used
    - Although it is advised to use LC method as first choice
  - New issues covered
    - Reanalysis
    - Endogenous compounds analysis
  - Criteria for application of a validated method
  - Workshops between ACBIO and COBIO/ANVISA for previous discussions

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Brazilian Guideline on BMV

- RE899/03
  - Validation Parameters
    - Specificity
    - Calibration curve/Linearity
    - Precision (HQC, MQC, LQC) – intra and inter-run
    - Accuracy (HQC, MQC, LQC) – intra and inter-run
    - Lowest Limit of Quantification (LLOQ)
    - Detection limit
    - Recovery
    - Stability
      - Freeze-and-thaw
      - Short-term
      - Long-term
      - Post-processing
      - Stock solution
      - Comparison against freshly prepared samples

- CP33
  - Validation parameters
    - **Selectivity**
    - Calibration curve
    - Precision – including Dilution integrity (LLOQ, HQC, MQC, LQC, DQC)
    - Accuracy - including Dilution integrity (LLOQ, HQC, MQC, LQC, DQC)
    - **Carry over**
    - **Matrix effect (Matrix factor)**
    - Stability
      - Freeze-and-thaw
      - Short-term
      - Long-term
      - Post-processing
      - Stock solution
      - **Comparison based upon nominal conc.**

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Topics included into the revised version

- Selectivity
  - Biological matrix
    - Plasma: 6 sources (including hemolized and lipemic)
    - Whole blood: 5 sources (including lipemic)
    - Other: it should evaluated and tested
  - How to determine hemolysis?
    - Each lab should have a SOP
  - Co-medications/metabolites should be also tested (if necessary)
- Carry over
  - Inject ULOQ followed by 3 blank samples
  - Same criteria as in selectivity

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Topics included into the revised version

- Matrix effect
  - Expressed IS normalised matrix factor for LQC and HQC
  - Bilogical matrix:
    - Plasma: 8 sources (4 normal, 2 lipemic and 2 hemolysed)
    - Whole blood: 6 sources (4 normal and 2 lipemic)
    - Other: 6 sources
  - For each source should be calculated the IS normalized MF as:

$$\text{IS MF} = \frac{\dfrac{\text{Analyte response matrix}}{\text{IS response matrix}}}{\dfrac{\text{Analyte response solution}}{\text{IS response solution}}}$$

    - CV <15%

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Topics included into the revised version

- Calibration curve
  - Use the same biological matrix of the study
  - Non-linear response should be justified
    - Linear: the weight of the equation used should be justified
  - Criteria
    - 75% of all replicates should pass (±20% for LOQ and ±15% for others)
    - At least 6 standards (8 for non-linear) should be included

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Topics included into the revised version

- Precision and accuracy
  - QC at 3 levels: Low, medium and high
  - LLOQ
  - Diluted QC for dilution integrity purposes
    - Should cover the expected dilution of the study samples
  - 5 replicates (within-run) and 3 runs (between-run)
  - Precision
    - CV% <15% (20% for LOQ)
  - Accuracy
    - Calculated as (mean / nominal conc. X 100)
    - 115 – 85% (120 – 80% for LOQ)
    - ACBIO suggested to change the way to calculate this parameter as relative error ([mean – nominal conc.]/nominal conc. X 100)

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Topics included into the revised version

- Stability
  - 3 samples in 2 levels (LQC and HQC)
  - Freshly prepared calibration curve
    - Compare with nominal conc.
      - Not compare with freshly prepared samples (major change)
    - Processed sample or on-instrument, Short-term, long-term and freeze-and-thaw
    - Criterion: ±15% of nominal conc.
    - Stock and working solution
      - Compare in response (area)
      - Criterion: ±10%

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Topics included into the revised version

- Endogenous compounds analysis
  - Neat solution (instead of biological matrix)
  - Surrogate matrix
  - Generic approach comparing the slopes of the curves
- Reanalysis
  - Accepted only in 3 cases
    - 1) Concentration above ULOQ (dilution needed)
    - 2) Analytical problems (poor chromatography, injection fail, etc.)
    - 3) Pre-dose samples with concentration above LLOQ
  - PK reason is not accepted
  - CC and QC samples should not be reassayed

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Issues not covered

- Ligand Binding Assays (LBA)
  - Incipient in Brazil under GLP conditions
  - ANVISA reported only few applications using this technique
- Incurred Samples Reanalysis
  - ANVISA is trying to better understand the real purpose of this test
  - In case of fail, what to do with the data?
- IS variation
  - Still not have consensus on how to deal with this issue

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Conclusion

- Brazilian BMV guideline is becoming more comprehensive
- ANVISA is trying to adopt international practices in bioanalysis
  - "ACBIO-Bioanalises" is contributing in this process
- ACBIO-Bioanalises in trying to put together all bioanalyst for pursuing this goal
  - ANVISA is open for harmonization process
- Once published the final version of the ANVISA BMV guideline ...

1st in South Hemisphere?

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- 1st Latin American Meeting on Bioanalysis
  - May, 2012
  - São Paulo/SP
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