ANVISA's Bioanalytical Guidance

RDC 27/2012

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Sumary

- Review process of RDC 899/2003
- New Brazilian guidance and the EMA 2011 guidance.

Review process of RDC 899/2003

- Separation between the analytical methods guidance and bioanalytical methods guidance.
- Creation of a working group on November 2010
- Discussion of the first proposal in a workshop sponsored by the Brazilian Association of Centers of Bioavailability and Bioequivalence of Medicines (ACBIO-BR)
- Consultation of the Draft from 06/30/2011 to 08/29/2011 (PC 33/2011)
- Publication of RDC 27 on may 2012.

New Brazilian guidance and the EMA 2011 guidance

Scope

ANVISA		EMA
Bioanalytical methods used in studies for market authorization of medicines.		Methods applied to measure drug concentrations in biological matrices obtained in animal toxicokinetic studies and all phases of clinical trials.
Any analyte in a biological matrix		Biomarkers used in assessing pharmacodynamic endpoints are out of the scope.
No specific tests for ligand binding assays. Allows adaptations.		Ligand binding assays separated from chromatographic methods.

Full, partial and cross validation

ANVISA		EMA
A full method validation should be performed for any analytical method		
Whenever changes occur in a method, full validation or partial validation should be performed, according to the relevance of the modification.		When minor changes are made, a full validation may not be necessary, depending on the nature of the applied changes.
When the impact of the change is unknown, full validation should be performed.		Give some examples of changes that need partial validation
		Cross validation

Reference Standards

ANVISA		EMA
Compendial standards. Characterised standards.		Compendial standards. Commercially available standards. Sufficiently characterised standards prepared in-house or by an external non-commercial organisation.
Certificate of analysis stating: identity, content, and date of expiration or retest		Certificate of analysis to ensure: purity and provide information on storage conditions, expiration date and batch number of the reference standard.
Documents issued by the manufacturer containing: Nomenclature, CAS; chemical name; synonymy; molecular formula and structure, molecular weight, physical form; physicochemical properties; impurity profile; care and handling and storage		
The use of certified standards is not needed for IS		

Selectivity

ANVISA		EMA
6 individual sources of the appropriate blank matrix		
Response less than 20% of the lower limit of quantification for the Analyte and 5% for the Internal Standard		
Plasma: 4 normal samples, 1 hyperlipidaemic and 1 hemolyzed Blood: 5 normal samples and 1 hyperlipidaemic		

Carry-over

ANVISA		EMA
3 injections of the same blank sample, 1 before and 2 after the injection of one or more samples of processed LSQ.		Injecting blank samples after a high concentration sample or calibration standard at the upper limit of quantification.
Should not be greater than 20% of the LLOQ signal and 5% for the internal standard		
If it appears that carry-over is unavoidable specific measures should be considered		

Matrix effect

ANVISA		EMA
At least 6 lots of blank matrix		
Individual sources		Individual donors
The CV of the IS-normalised MF calculated from the 6 lots of matrix should not be greater than 15 %. This determination should be done at a low and at a high level of concentration		
Plasma: 4 normal samples, 2 hyperlipidaemic and 2 hemolyzed Blood: 4 normal samples and 2 hyperlipidaemic		Recommend to investigate matrix effects on other samples e.g. haemolysed and hyperlipidaemic plasma samples, special populations, excipientes.
If this approach cannot be used, for instance in the case of on-line sample preparation, alternative procedure is accepted		

Calibration curve

ANVISA		EMA
A minimum of 3 curves should be reported		
A minimum of six concentration levels should be used, in addition to the blank and a zero sample		
Scientific justification for the concentration range should be presented		Ideally, before carrying out the validation of the analytical method it should be known what concentration range is expected
A relationship which can simply and adequately describe the response of the instrument with regard to the concentration of analyte should be applied		
Back calculated concentrations should be within ±15% of the nominal value, except for the LLOQ for which it should be within ±20%. At least 75% of standards, with a minimum of six calibration standard levels, must fulfill this criterion		

Accuracy and Precision

ANVISA		EMA
Incorporates Dilution Quality Control		Specific topic for Dilution Integrity

Stability of the analyte in the studied matrix

ANVISA		EMA
Evaluated using low and high QC samples which are analysed immediately after preparation and after the applied storage conditions that are to be evaluated.		
The QC samples are analysed against a calibration curve, obtained from freshly spiked calibration standards. The mean concentration at each level should be within ±15% of the nominal concentration		
Freeze and thaw; short term stability; long term stability.		
Stability of the processed sample.		Stability of the processed sample under storage conditions. On-instrument/ autosampler stability .

Stability of the stock and working solutions

ANVISA		EMA
Tested with an appropriate dilution, taking into consideration the linearity and measuring range of the detector		
It is not needed to study the stability at each concentration level of working solutions		
It is not needed to study the stability of stable-isotope labelled internal standards if it is demonstrated that no isotope exchange reactions occur under the same conditions		
The mean instrumental responses from the solutions under study should be compared with the mean of those obtained using freshly prepared solutions of the analyte and the IP		
Deviation < 10%		

When the matrix free from analyte is not available

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Selectivity can be tested by comparing the inclinations of at least 6 standard added curves in 6 samples of different sources of biological matrix (containing a basal level of analyte) and the standard curve in solution or surrogate matrix
Validation of the calibration curve, accuracy and carry-over can be performed using calibration standards and QCs in solution or replacement matrix.
Validation of precision and stability studies should be performed in the same matrix of study samples.
I - comparing the concentrations obtained from freshly prepared samples with those obtained from the same samples after a period of stability study, or II - comparing to the nominal values , since the matrix is previously analyzed and supplemented in order to achieve concentrations of the LQC and HQC.

Analytical run

ANVISA		EMA
An analytical run consists of the blank and a zero sample, calibration standards at a minimum of 6 concentration levels, at least 3 levels of QC samples in duplicate or at least 5 % of the number of study samples, whichever is higher, and study samples to be analysed.		
All samples should be processed and extracted as one single batch		
For bioequivalence all samples of one subject should be analysed together in one analytical run. Exception for reanalysis		For bioequivalence it is advised to analyse all samples of one subject together in one analytical run.
QC samples should be distributed among the study samples in a balanced way, in the same number of replicates of each concentration		The QC samples should be divided over the run in such a way that the accuracy and precision of the whole run is ensured
At least 2 QC sample levels should fall within the range of concentrations measured in study samples.		
Dilution QC		

Acceptance criteria of an analytical run

ANVISA		EMA
Back calculated concentrations of the calibration standards ±15% (±20% for LLOQ). At least 75%, with a minimum of six, must fulfill this criterion.		
If the rejected calibration standard is the LLOQ, the LLOQ for this analytical run is the next lowest acceptable calibration standard of the calibration curve.		
QC samples should be within ±15% of the nominal values. At least 67% of the QC samples and at least 50% at each concentration level, must fulfill this criterion		

Reanalysis of study samples

ANVISA		EMA
Reasons for reanalysis of study samples and criteria to select the value to be reported should be predefined in the protocol, study plan or SOP		
For bioequivalence studies should not be performed reanalysis for pharmacokinetic reasons		For bioequivalence studies, normally reanalysis of study samples because of a pharmacokinetic reason is not acceptable.
situations in which the samples should be reanalysed		examples of reasons for study sample reanalysis

Scope

ANVISA		EMA
Concentration above the ULOQ or below the run's LLOQ, in runs where the lowest standard sample has been rejected from a calibration curve, resulting in a higher LLOQ compared with other runs,		
Analytical problems that preclude or invalidate the quantification		Internal standard response if criteria have been pre-defined; improper sample injection or malfunction of equipment poor chromatography
Identification of quantifiable analyte levels in pre-dose samples or placebo sample		

Incurred samples reanalysis: Whay it was not mentioned in the RDC 27/2012

• The use of calibration standards and QC samples during validation may not mimic the actual study samples.

• This kind of reproducibility indicator could substitute some structural requirements for CROs certification.

•There was relevant publication about the topic since 2007.

But for a guidace for minimum requirements we believe that the answers of those primary questions should be more mature.

- In what type of studies should samples be reanalyzed? (bioequivalence?)

- What constitutes acceptable reproducibility and in what manner should the data be analyzed to arrive at valid conclusions?

- What actions, if any, should be taken, once the analysis is completed, and the results have been evaluated?

Mario L. Rocci, Jr., Viswanath Devanarayan , David B. Haughey , and Paula Jardieu. *Confirmatory Reanalysis of Incurred Bioanalytical Samples*. The AAPS Journal 2007; 9 (3) Article 40.

Thank you