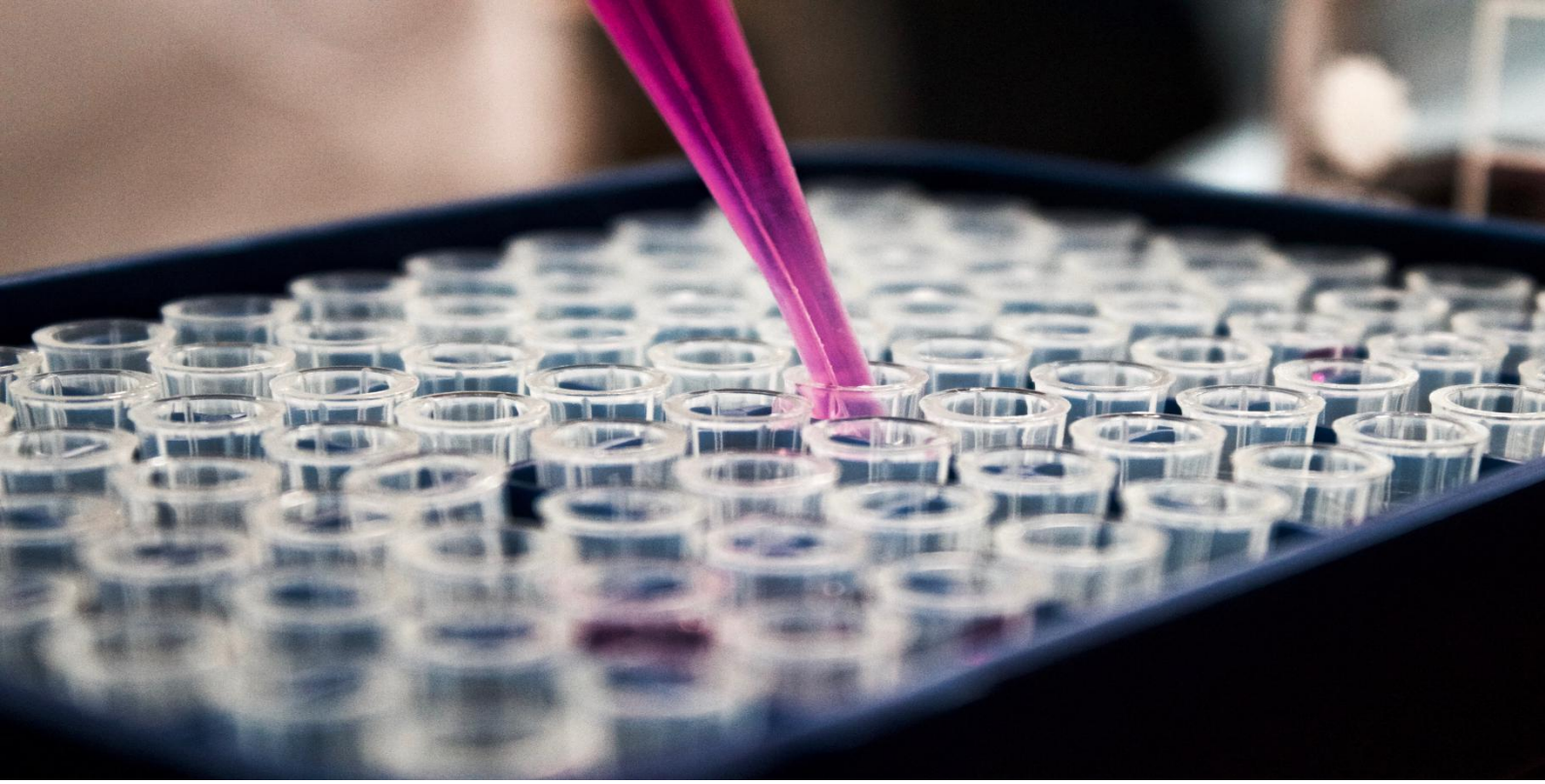

Bioanalytical Method Validation FDA 2018
- A Practical Assessment -

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In May 2018 the US Food and Drug Administration issued new guidelines on the validation of bioanalytical methods used in human clinical pharmacology, bioavailability and bioequivalence studies.

Now that a year has passed we can look back and make a practical assessment of the implications this guideline has brought to industry, and the pitfalls or benefits for pharma companies following it. The goal of this article is to summarize the important points which should be taken into consideration.

"The guideline is comprehensive and compact"

The guideline is comprehensive and compact (the text contains only 18 pages) and might be used for nonclinical studies as well. Although the period between the last FDA guideline (2001) and the issuance of the new one in 2018 seemed to be extremely long, during that time there were tremendous advancements in

bioanalytical method development and the arrival of several new workshops partially compensated this high need.

"..the FDA is open to use other approaches and encourages the development of new bioanalytical technologies.."

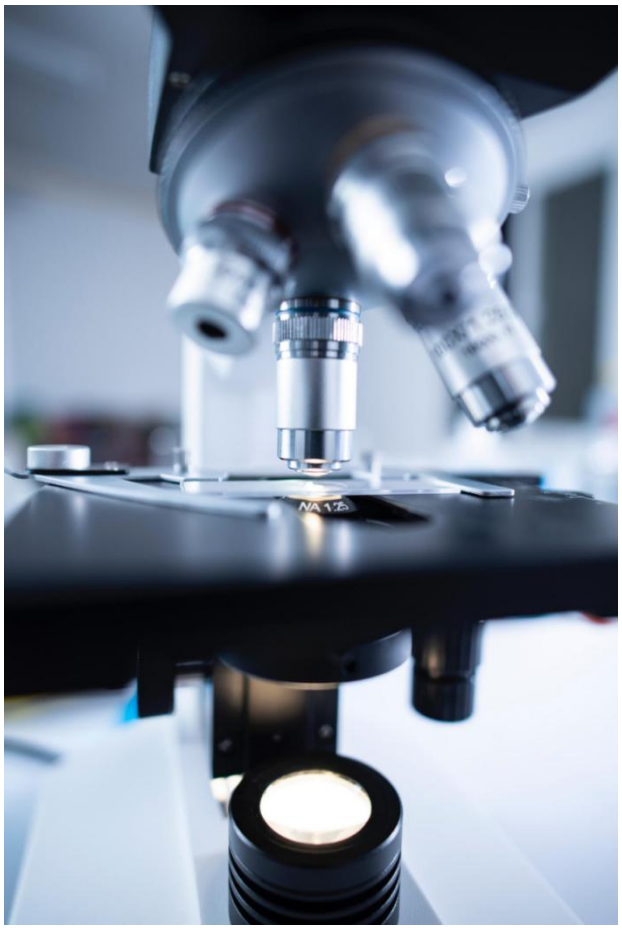
Also, during that time in 2012, the EMA's guideline on bioanalytical method validation went into effect. The new guideline contains nonbinding recommendations and the meaning of the word "should", as frequently used in the guideline, is defined as "something is suggested or recommended but not required". Thus, the FDA is open to use other approaches and encourages the development of new bioanalytical technologies like Dried Blood Spots, etc., (cf. V. D, page 17), assuming the data are bridged to that of other method. The scientific and professional approach is emphasised as very important. Therefore, it appears that the gap of 17 years between the previous and new FDA guidelines seems to not be critical.

Turning our focus now to method validation, the tables in the Appendix VII. summarize all acceptance criteria for method validation as well as in-study conduct. This will probably be the most frequently used part of the guideline. Worth noting is that essentially there are almost no differences in the recommendations and acceptance criteria between FDA and EMA. Both are considering all important bioanalytical parameters to be evaluated in the validation process, except of recovery. In fact, contrary to the FDA guideline the EMA guideline does not consider the recovery as an important parameter at all.

For the first time, the FDA has provided a correct definition of recovery (excluding the influence of matrix effect). This could be considered an essential qualitative improvement. However, there are inconsistencies between the same criteria for

accuracy and **precision** used in the validation and the accuracy for individual QCs in study analyses. Nevertheless, these inconsistencies exist in all present guidelines.

The acceptance criteria for QCs (for chromatographic assays - CC) are following: ≥ 67% of QCs should be ± 15% of the nominal, and ≥ 50% of QCs per level should be ± 15% of their nominal. On the other hand the same criterion is required for precision in validation defined by the statistical term as ± 15% CV. According to the error distribution function, approximately 68% of all individual QC values obtained are within the interval ± 15% CV, thus, 32% of the individual values in study analyses would not meet the acceptance criteria.



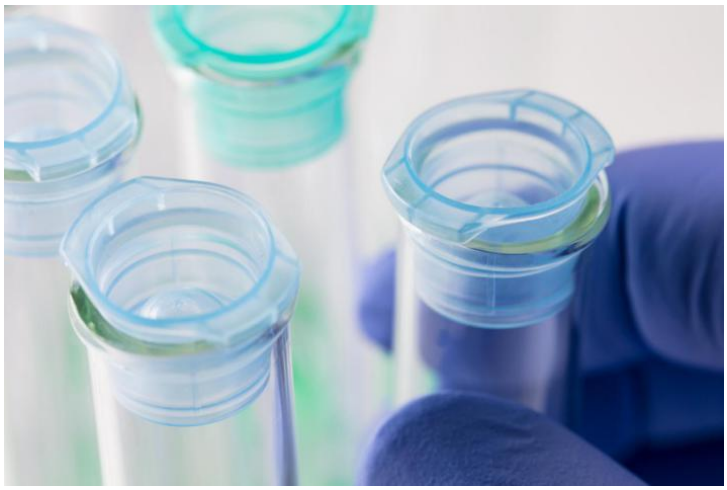
Assuming the model where the accuracy of the method is ± 0% of nominal concentration (100% accuracy) at all 3 QC levels, and the precision obtained in the validation process is ± 15% CV, then the probability of the run rejection as defined above would be about 36%. This is very high probability, and frequent run fails could be considered unacceptable.

For a more realistic model let's place the accuracy of the method ± 10% of nominal concentration at all 3 levels, and the precision ± 7.5 % CV. Now in the validation process the evaluated probability of run rejection is 23%, which is still a relatively high value.

Therefore, in order to avoid a high number of rejected runs it is recommended to use stricter criteria for accuracy and precision. For example, the limits for precision and accuracy ± 12% for LLOQ and ± 8% for other concentration levels of standards, as well as for all QCs, might be adequate for validation criteria.

Selectivity and Specificity:

Depending on the intended use of the assay, effect of haemolyzed samples, lipemic samples or samples from special populations **can be included** in the selectivity and matrix effect assessment. The EMA guideline strictly recommends (requires) to investigate influence of such special samples, especially in matrix effect assessment. This is an illustration of the main difference between the FDA and EMA approaches. FDA prefers a less strict and more scientifically based approach.



According to the guideline the sponsor **should ensure** that **there are no matrix effects throughout the application of the method.** This requirement may hardly be achievable, both practically as well theoretically (see e.g. M. Ryska, Eur. J. Mass Spectrom. 21, 423-432 (2015)). When using LC/MS methods, the sponsor should determine the effects of the matrix with regards to ion suppression, ion enhancement, or extraction efficiency. The problem, however, is that no method for matrix effect determination is given. The method for matrix effect evaluation and acceptance criteria required by EMA guideline are more advanced in this respect. Therefore, it can be concluded that the EMA approach is preferred as the more sophisticated one.

"Therefore, it can be concluded that the EMA approach is preferred as the more sophisticated.."

The paragraph "Validated Methods: Expectations and In Study Analyses and Reporting" (III. C, page 12-13) is comprehensive and thoroughly described. It does not need any additional suggestions or comments.

The recommended acceptance criteria for ISR (page 27) ± 20% (or ± 30% for LBA – ligand binding assays) **of the mean** might be slightly misleading. The difference

between two values (original and ISR value) was meant to be within 20% of their mean value as given by formula on page 15.

Stability

Acceptance criteria: The accuracy (% nominal) at each level should be ± 15%. Such criteria might be not sufficient for the real stability presentation. For example, if accuracy at the starting point would be **+ 15%** and the one at the last point of evaluation **– 15%**, the criteria still would be met but the real instability (decrease of the analyte concentration) might be up to -30%. Thus, it is to recommend that parallel to the presentation of accuracy as % of nominal, the relative concentration as % of starting point and trends graphs should be presented as a more adequate assessment of stability data.

Determination of stability at -20^{o}C would cover stability at colder temperatures, and a scientific approach such as this is appreciated. The EMA recommendation requires to determine the stability at two temperatures thus to cover the temperatures between such two limits, but that approach could be considered useless.

Comprehensive and concrete requirements on Documentation and Reporting are given on table 2 of the Guidance. These are to be appreciated by all sponsors or applicants.

Conclusion

The new FDA guideline encourages applicants to use a rather more scientific approach for methods development and their validation, versus the corresponding EMA one. Tables summarising both acceptance criteria for validation parameters as well as requirements on Documentation and Reporting are very much appreciated. We believe that in spite of its non-binding character, it will be a practical guideline in each bioanalytical laboratory. And lastly, in light of the fact that the new ICH guideline draft has recently been published for comments, it should be interesting to see what the similarities and differences between these two guidelines will be at the end.

We will review the new guideline and share our analyses and conclusions with you afterward.

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