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COMMENTARY

Bioanalytical Method Development and Validation: from the USFDA 2001 to the USFDA 2018 Guidance for Industry

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Introduction

Recently, the USFDA issued the new 2018 guidance document for industry on bioanalytical validation. Due to this occasion, it would be worthy to look back in time as well into the (near) future on potential practical impacts the 2018 guidance document might have on bioanalytical method validation and laboratory operations. Bioanalytical method development and validation is the most important part in regulated bioanalysis. Validated bioanalytical methods are used for the quantitative measurement of drugs and their metabolites, endogenous compounds, and biomarkers in biological fluids. Drug concentrations are indispensable for the evaluation and interpretation of pharmacokinetic (PK), toxicokinetic (TK), and bioequivalence (BE) study data. The bioanalytical methods are not only applied for quantification of small molecules (molecular weight ≤ 900 Da) but also for larger molecules such as proteins, antibodies, and peptides. Bioanalysis can be quite challenging due to the complexity of the biological sample matrix. In addition to the sample complexity, data quality obtained from analyzed samples is directly related to the bioanalytical method's performance. Without any doubt, it is of utmost importance that bioanalytical methods used in bioanalysis have to provide reliable data.

According to the USFDA is the purpose of bioanalytical method validation: 1) to validate operation conditions, limitations, and 2) to determine the method suitability for its intended purpose and 3) to ensure that the bioanalytical method is optimized for sample analysis.

For a long time, method validation procedures and strategies used in bioanalysis, as well as acceptance criteria needed for validation procedures, were a matter of personal prejudice. Many years there existed a lack of guidance uniformity on bioanalytical method development and validation within the bioanalytical community. This suddenly changed when industrial committees and regulatory agencies initiated the development and introduction of guidance documents on bioanalytical method validation for industry [1].

The first USFDA guidance document for industry on bioanalytical method validation was issued as a draft guidance in January 1999. Two years later this draft guidance document was finalized after incorporation of public comments, and the guidance was released as an official guidance document in May 2001. The guidance docu-

ment text was based on deliberations of two workshops jointly organized by USFDA, and American Association of Pharmaceutical Scientists (AAPS) and other institutions [2,3]. This guidance document provided assistance to sponsors on bioanalytical method validation for Investigational New Drug (IND) Applications, New Drug Applications (NDA), Abbreviated New Drug Applications (ANDA). Additionally, the guidance document also guided the development and validation of bioanalytical methods applied in pharmaceutical- and clinical studies in research areas such as in clinical pharmacology, bioavailability, and for BE studies. This 2001 guidance document was many years one of the few and without any doubt one of the most used bioanalytical method validation guidance documents for industry among other guidance documents from ICH, IUPAC, and USP. The 2001 guidance document was specifically created as guidance for the validation of bioanalytical methods using instrumental analytical techniques such as gas chromatography (GC), high-pressure liquid chromatography (HPLC), both with regular detectors or in combination with mass spectroscopy (MS) as well as also for immunological and microbiological methods. The 2001 guidance document was updated in September 2013 after two additional workshops organized in 2006 and 2008 by FDA and AAPS [4]. The 2013 guidance document was issued as a draft guidance document for the general public to review before it would be finalized. In contrast to the 2001 guidance document, the 2013 draft guidance extended its scope for also Biological Licence Applications (BLA) by the introduction of four new sections. The 2013 draft guidance provided next to chromatographic and immunological methods now also assistance on the validation of bioanalytical methods for the analysis of endogenous compounds, biomarker analysis, and for the application of diagnostic kits and new technologies. Identical to the 2001 guidance document, the recommendations are given for bioanalytical method validation were also in the 2013 draft guidance document non-binding and deviations from the guidance by applying other or different validation approaches were allowed depending on the bioanalytical method type used.

2018 USFDA Guidance for Industry on Bioanalytical Method Validation

In May 2018, the USFDA released a new guidance document for industry on bioanalytical method validation. Since the release of the 2013 draft guidance document, a finalization of this guidance document was highly anticipated for many years by the bioanalytical community [5]. After studying the recently released USFDA 2018 guidance document [6], we decided that some considerations should be presented in the present article. Nevertheless, it must be addressed that the presented considerations should not be seen as any additional recommendations or deviations to the present guidance document. The hope exists that present article, will to some extent generate discussions on how the new 2018 guidance document may impact current and of course also future bioanalytical method validation practices and standard operating procedures (SOPs). What can be noticed is the overall improvement of the readability and the well-organized structure of the new guidance document over the 2013 draft guidance document. Not only the improved readability but also a more better reflected logical flow of the different presented validation sections can be noticed.

Introduction Section

In the Introduction section (Section I), the guidance document quotes “recommendations can be modified with justification, depending on the specific type of bioanalytical method”. This statement is to inform the reader and/or user of the guidance document that one can deviate from recommendations presented in the guidance document but that a justification for the applied deviation must be given. It is a kind of different approach the USFDA chooses with this new guidance document compared to the 2013 draft guidance document. In the 2013 draft guidance, a justification for deviation from the guidance document was not mandatory.

Background Section

In the Background section (Section II), typical key questions are presented one has to address when a new method has been (fully) validated. Important questions like: "Does the method measure the intended analyte?", "What is the variability associated with these measurements?" and also other questions are presented. These questions were added to the present guidance document after being absent in the 2013 draft guidance document. From our point-of-view, these questions are very helpful in the evaluation of the selected bioanalytical method validation design by recapturing thoughts and giving the possibility to brainstorm about (potential) important validation parameters and pre-analytics.

Moreover, in this section, the fit-for-purpose (FFP) concept has been clarified. The FFP concept expects that a full validation should be applied to "pivotal studies submitted in an NDA, BLA or ANDA that require regulatory decision making for approval, safety or labeling, such as BE or pharmacokinetic studies. In general, the FFP concept is to establish the purpose of the bioanalytical method and evaluate as well agree on validation execution level (fully or not fully validation) that should be appropriate for the intended purpose of the new bioanalytical method. From the definition of this concept it can be concluded that for exploratory methods that are not planned to be used to support regulatory decision making, a validation design according to an FFP would be sufficient and satisfactory. This conclusion is confirmed in the 2018 guidance document by stating that for exploratory methods that are not used to generate data for supporting regulatory decision making that the need for a stringent validation (full validation) may not be required. This will create the possibility as also the freedom to choose between a full validation or a FFP validation for a new developed bioanalytical method. Confusion on applying the FFP concept can arise according to the present guidance document when the FFP concept has to be used for drugs, their metabolites, and biomarkers. What about endogenous compounds that are not designated as (potential) biomarker, but could be of interest to be validated? Should the bioanalyst involved in bioanalytical method validation have to perform a full method validation by first consulting with FDA to confirm whether the assay needs to be fully validated?

Bioanalytical Method Development and Validation Section

In this section (Section IIIA), stipulations around understanding the physicochemical properties from the analyte of interest, confirming the identity of reference standards as well as information on drug metabolism and protein binding are necessary prior to the development of a bioanalytical method. Furthermore, the present guidance document delivers an enhanced focus on method development designs by presenting a comprehensive outline of bioanalytical parameters that should undergo optimization of procedures and conditions to ensure that the bioanalytical method is suitable for validation. In contrast to the 2013 draft guidance document, the list was extended in present guidance document with the following bioanalytical parameters: reference standards, critical reagents, calibration curve, quality control samples and recovery. This list demonstrates that consciousness on the number of important bioanalytical parameters before the start of the validation experiments increased. We think that we speak on behalf of many bioanalytical scientists saying that the importance of many of the bioanalytical parameters was self-evident already before the 2018 guidance document listed them.

The bioanalytical validation parameters that are recommended for chromatographic assays (CCs) and ligand binding assays (LBAs) are now structured presented as a table (Table 1 in the Appendix (Section VII). In the present guidance document, both analytical technologies are merged into one single section presenting all different validation parameters. This merging of the two different analytical technologies and their corresponding acceptance criteria into one section has made the guidance document from our point of view more user-friendly as well and better structured.

In section Selectivity and Specificity (IIIB.4), the validation of bioanalytical methods when using LC-MS, recommendations on the determination of ion suppression or ion enhance-

ment effects are presented. Recommendations on how the experimental procedure should be designed for the determination of these typical effects familiar to LC-MS analysis are missing, this in comparison to the EMA guidance document on bioanalytical method validation where a protocol is described [7]. Furthermore, the bioanalytical method's selectivity should be demonstrated for new methods by analyzing blank samples from the appropriate biological matrix collected from multiple sources. This recommendation could become critical or difficult to be applied in the validation practice for the validation of bioanalytical methods on endogenous compounds and biomarkers since the availability of analyte depleted biological matrices are very limited or very expensive.

In the subsection Accuracy, Precision and Recovery (Section IIIB.6) recommendations on the evaluation of the analyte recovery are presented by the comparison of extracted samples with corresponding extracts of blanks spiked with analyte post-extraction. This is a very common daily practice for the determination of recovery rates for analytes but what if the analyte of interest is an endogenous compound or (potential) biomarker and when the nominal concentration is unknown, below LLOQ or difficult to determine by spiking? The present guidance document does not give any recommendation neither in this section nor in the successive sections on endogenous compounds and biomarkers. The determination of analyte recovery is a frequently observed critical issue in the validation of endogenous compounds and biomarkers. Moreover, the use of freshly prepared calibrators and QCs for accuracy and precision determination especially for runs over several days could be from a practical point of view complicated and it would increase the workload during method validations.

The present guidance document recommends the study of analyte stability in the presence of other drugs or co-medications that are known to be administered regularly. One could question if the stability of the analyte would change dramatically due to the co-administration of other drugs and what would be the underlying process. Can we not generally assume that the rate of drug metabolism would in nearly all cases impacts the "stability" of the analyte significantly more than the co-administered drugs would do?

The subsection presents recommendations on the validation of dilution integrity by using QC samples above the ULOQ of the bioanalytical method. Also in this section of the guidance document recommendations on the validation of the dilution integrity for endogenous and biomarker assays are lacking.

In section IIIC, the application of the validated bioanalytical methods for in-study analysis and reporting are presented. There are some highlights worth noting. We think that the requirement to report batch performance criteria could require many laboratories working with 96-well plates for some change of procedure in handling QC samples.

The recommendation of using QCs interspersed with study samples is not a very common way QC samples are analyzed during runs, many laboratories are using the bracketed use of QCs with study samples and many will probably keep using the bracketed way of including QCs into study sample runs.

A new concept for reporting an analyte concentration below the lower level of quantitation (LLOQ) is presented in this section. An analyte concentration below the LLOQ should from now on be listed as below LOQ (BQL). The 2013 draft document recommended reporting these analyte concentrations to be reported as zero concentration, although they are per definition not zero but just below the LLOQ, this way of reporting a concentration below LLOQ is now fortunately and correctly replaced by the definition below LLOQ (BQL).

Incurred Sample Reanalysis (ISR) Section

This validation parameter is in present guidance document presented as a separate section due to heightened awareness and precedence set by other BMV guidance documents. The only notable update compared with the 2013 draft guidance is the manner of ISR sample selection. The number of samples required has been changed in 10% of the first 1000 study samples with an additional 5% of ISR samples for the remaining number

of study samples. The ISR of study samples could become critical when it is not exactly known how many study samples are expected or when inclusion of study subjects is ongoing or difficult to predict. Also noted is that the ISR sample selection of samples should be around Cmax and from the elimination phase, a selection design for ISR samples that is from our point-of-view in conflict with a truly statistical random sample selection.

Additional Issues Section

The section on Additional Issues (Section V) remained from the 2013 draft document but in the present guidance document, several important updates can be noted. The section on Endogenous Compounds section has been consolidated by the addition of one single bullet point recommending the study of parallelism. Parallelism is a test for demonstrating potential matrix effects by using a serial dilution of (incurred) study samples. Furthermore, it is recommended that the biological matrix used to prepare calibrators should be the same matrix as the study sample's matrix and free of endogenous analyte. From our point of view is the preparation of calibrators in a biological matrix free from analyte in many occasions difficult or shear impossible, due to the very limited availability of analyte depleted biological matrices, especially in the case of the validation of endogenous compounds or (potential) new biomarkers.

The QC samples for the validation of endogenous compound assays should be prepared by spiking the analyte into the biological matrix. This could result in some critical problems especially for the low QCs with the determination of their nominal analyte concentration since the background concentration of the endogenous analyte is unknown and could be difficult to determine. How to proceed when the concentration of the endogenous compound is below the lower limit of quantification? Moreover, the determination of the recovery rate for endogenous compounds is difficult to determine, so that in conclusion only an estimation of the concentration of matrix-matched QC samples is possible.

The reference to parallelism also extends to the next sub-category within the additional section on biomarkers. The application of an FFP approach for the determination of the appropriate extent of bioanalytical method validation is recommended unless when the obtained biomarker data is used to support a regulatory decision such as pivotal determination of safety and/or effectiveness or to support dosing instructions in product labelling, a full validation becomes necessarily. It's worth pointing out that the application of this guidance document for the validation of biomarkers remained unchanged compared to the 2013 draft document and we do hope that (a) future guidance document(s) the assistance/guidance for the analysis of biomarkers will become more elaborated and validation design clarified.

The section V.C on the use of diagnostic kits was significantly rewritten. An earlier comment in 2013 draft document stating that the recommendations given in this sections do not apply to Clinical Laboratory Improvements Amendments (CLIA) activities has been removed in the present guidance document. Besides the use of diagnostic kits for PK and PD studies the 2018 guidance document includes now also biomarkers in this section and we are curious how and where interpretations of present guidance will take us regarding the validation of biomarkers when using diagnostic kits in a clinical laboratory setting.

The New Technologies section F has been divided into two separate sections in the 2018 guidance document; Bridging Data from Multiple Bioanalytical Technologies and Dried Blood Spots (DBS). The present guidance gives recommendations on when a new platform is used that generates drug concentrations that are different from another platform, how this issue should be taken care of. Furthermore, assistance on how bridging the data produced by new technology platforms is presented. The bridging should include the application of incurred samples and in case a bias is observed in the correction of concentrations by use of mathematical transformation by a factor is allowed. In contrast, to this statement, the use of two different methods for BE studies in ANDAs is discouraged by the guidance document without any explanations.

What stands out from our point of view in the present guidance document are the recommendations from the DBS section on requirements for correlative studies with traditional sampling during drug development when DBS is used. It is not clear if the example of DBS in present guidance document is used to stimulate discussions, although the application of DBS in drug development is discussed for many years now, or on how to adapt and validate new technologies other than DBS like several other microsampling techniques and/or the use of alternative biological matrices such as saliva, urine, hair, and stool etc.

Documentation and Reporting Section

This section is significantly consolidated compared to the 2013 draft version. With the inclusion of Table 2 in the Appendix it helps to clarify certain requirements for documenting and reporting. Depending on existing report templates, the stipulated requirements for the final report might lead to some significant changes to current validation and study practices for laboratories. The introduction of BQL could cause potentially some reporting issues not only for the data management but also for customers due to the introduction of this new terminology. The presentation as well explanation of new terminology for analyte concentrations below the LLOQ (e.g. < 4 mg/dl) could require in some cases a time-consuming investment into the education of customers and adjustment of data management on presenting results from (clinical) studies using this new terminology. It may seem inconvenient to adapt, but report as BQL with a defined LLOQ is a better practice.

Conclusion

The bioanalytical community has been expecting the present guidance document for industry on bioanalytical method validation for quite some years. The draft guidance document was under review for approx. 5 years, and we are convinced that the bioanalytical community will embrace the 2018 guidance document, in the same way as the community, did with the 2001 and the 2013 draft guidance document. The fundamentals on bioanalytical method validation presented in the 2018 guidance document are quite consistent with the other guidance documents on bioanalytical method validation such as the EMA guidance document that has been acting as a reference document for regulated bioanalytical activities since its release by the European Medicine Agency in 2012. The sections on endogenous compounds, new technologies and biomarkers present some new recommendations but a complete revised assistance on how to validate these special assays are also in the present guidance document still not presented. We see the need for guidance on how to validate biomarker assays, but the application of concepts for xenobiotics in chromatographic and ligand binding assays for the validation of endogenous compounds and/or biomarkers using all possible analytical techniques might not be possible in many cases. We had hoped for more detailed guidance on how to validate biomarker assays taken into account the different analytical platforms and methods available. In addition, a clear definition of biomarkers especially for the assessment of safety is still missing, e.g. are full validations for biomarkers like liver enzymes (ASAT, ALAT, GGT) expected? Here we had hoped for clearer definitions and for more specified recommendations on the validations, especially for assays other than chromatographic or ligand binding assays. In conclusion, the present guidance document is primarily addressed for full validations and pivotal PK/BE studies supporting safety, efficacy and labeling claims. In comparison to the 2013 draft guidance document the present 2018 guidance document has not been changed significantly, in general, the major change the present guidance document underwent was the wording of the different guidance sections. In the assistance on full validation of bioanalytical methods is where the new guidance document presents consistency with other guidance documents such as the EMA guidance document on validation of bioanalytical methods.

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