Q11. 分析単位内の繰り返し分析はどうすればよいか？

A11. 分析単位内の精度を1分析単位の結果から算出する場合は，少なくとも3回の繰り返し（n=3）が必要である．分析単位間の精度を算出する場合は，分析単位内では1回の測定でも許容される．また，分析単位内の繰り返し回数を2回以上として，分析単位内及び分析単位間を区別せずに分散分析などの手法を用いて評価してもよい．

Q12. トータルエラーを必要とする根拠は何か？

A12. 真度から100%を引いた値の絶対値は測定における系統的誤差（systematic error），精度は偶然誤差（random error）を反映し，トータルエラーの評価によって，各定量値の真値からの乖離やばらつきが大きくその信頼性に問題を有するような分析法を早期に排除することができる（DeSilva et al, Pharm. Res., 2003）．リガンド結合法では，クロマトグラフィーを用いる分析法と比較して，真度及び精度の許容範囲が広く設定されており，分析結果の信頼性を確保する上で，真度及び精度の双方が許容基準値に近い分析法を極力排除するために設定した．

Q13. 内因性物質とアミノ酸配列が同じ薬物を分析する場合，真度の評価の留意点はあるか？

A13. 代替マトリックスや内因性物質を除去したマトリックスを用いるか，除去せずにブランク試料の内因性物質濃度を分析した上で以下のいずれかの式より真度を算出する．

$$\text{真度}(\%) = \frac{\text{試料中薬物濃度定量値}}{\text{内因性物質濃度} + \text{標準物質濃度}} \times 100$$

$$\text{真度}(\%) = \frac{\text{試料中薬物濃度定量値} - \text{内因性物質濃度}}{\text{標準物質濃度}} \times 100$$

《希釈直線性》

Q14. 希釈直線性は希釈の妥当性とどう違うのか？

A14. 希釈の妥当性の評価は，希釈操作が定量値に影響を与えないことを確認するために実施するが，希釈直線性の評価は，希釈の妥当性に加え，フック効果またはプロゾーンの有無を確認するためにも実施する．

《クロスバリデーション》

Q15. 判断基準が「各濃度における平均真度が原則として理論値の±30%以内」となっている理由はあるか？

A15. 本ガイドラインでは分析法における平均真度において，理論値の±20%であることを求めている．クロスバリデーションにおいては，さらに室内及び室間再現精度の要素が加わることから，判断基準を30%とした．なお，同一試験から得られる実試料を複数の施設で分析する場合には，分析法バリデーションとは別に，実試料や標準物質の取扱いを当該分析実施に関する計画書または手順書で規定する等，実試料分析においても施設間差を最小限にすることの配慮が必要である．

《実試料分析》

Q16. 異なるプレートで測定された検量線を用いることは可能か？

A16. 原則として，プレートを用いる分析系ではプレートごとに検量線を作成する必要があるが，バリデーションにより妥当性が示されれば，異なるプレートで作成された検量線を用いて定量することも可能である．ただし，QC 試料はプレートごとに必要である．

Q17. 平行性（Parallelism）の評価は必要ないか？

A17. 実試料の希釈系列における用量反応曲線と検量線系列の用量反応曲線が平行であり，実試料の数段階の希釈における換算値に希釈倍率による差が認められないとき，平行性が成立していると定義される．本ガイドライン発出の時点では，平行性が成立しなかった事例，平行性不成立の原因，平行性の不成立が医薬品開発に与えうる影響の程度等について，国内外ともに十分な知見が蓄積され議論が成熟している状況ではないことから，必ずしもすべての分析について平行性を評価する必要はない．ただし，分析対象物質や分析法の特性，あるいは，医薬品開発の過程で集積されたデータから，平行性が問題になる可能性が疑われる際には，可能な範囲で科学的に妥当な評価を行い定量値への影響を考察すべきである．

《実試料分析における QC 試料》

Q18. プレート内の検量線及び QC 試料の配置に留意すべき点はあるか？

A18. プレート内の試料の配置により，分析結果に一定の傾向が生じることが避けられない場合には，分析結果への影響を最小化できるように，検量線試料，QC 試料，実試料の配置や，1 調製試料あたりの測定ウェル数に留意する．

《ISR》

Q19. トキシコキネティクス試験のISRはどのように実施したらよいか？

A19. トキシコキネティクス試験の場合には，1動物種，1マトリックスあたり，1回ISRを実施すれば良い．ただし，分析方法に変更があった場合，分析施設が変わった場合などは改めてISRを実施する．

トキシコキネティクス試験に先立って行われる用量設定試験等の非GLP試験から得られる実試料を用いて，分析法バリデーション試験の中でISRを実施する方法も認められる．ただし，この場合には，用量や投与方法等の試験デザインがGLP試験と同等であることが求められる．

Q20. 臨床試験において，ISRはどのように実施したらよいか？

A20. ISRは薬物動態を主要評価項目とする代表的な試験で実施される．分析法の妥当性を早期に評価するために，なるべく医薬品開発の早い段階で実施する．

マトリックスの組成に差があると考えられる被験者群の臨床試験においては再度ISRを実施する．また，生物学的同等性試験では，試験ごとにISRを実施する．

Q21. 臨床試験において，分析法バリデーションを行う際に既に臨床試験から取得した実試料が存在する場合には，それをISRの試料として利用できるか？

A21. 代謝物を分析対象物質として追加する場合やISRの基準を満たさず分析法を改良して再分析を行う場合等，分析法バリデーションを行う際に既に臨床試験から取得した実試料が存在する場合には，それをISRの試料として利用することができる．ただし，このような場合でも実試料の提供者への同意取得は必須であり，ISRの実施の手順等はあらかじめ定めておかなければならない．

Q22. ISR全体として判断基準を満たしている場合に，乖離度が±30%以内との判断基準を逸脱した個別の実試料について，再分析は必要か？

A22. ISRの目的は実試料を用いた分析法の妥当性の確認である．このため，個別の乖離度で±30%を超える実試料があった場合にも，全体としてISRの判断基準を満たしている場合には再分析を実施する必要はない．

Q23. ISRの結果は報告書のどこに記載すべきか？

A23. 実試料分析においてはISRを実施した場合には実試料分析報告書における分析法の妥当性に関する結果として，分析法バリデーションにおいてはISRを実施した場合にはバリデーション報告書におけるバリデーションの結果としてISRの結果を報告し，分析法の妥当性について考察をする．

Q24. 非臨床試験ではISRに供する測定試料が不足することが考えられるが，試料が不足した時の対応はどうすべきか？

A24. 非臨床試験においてもISRの実施を前提として試験を計画することが必要である．一部の時点の試料が再測定等で不足したとしても，別の時点の試料をISRに供する等，ISRを評価することは可能である．また，採取条件が同等である予備試験の試料を活用することもできる．非臨床試験においても，可能かつ適切な手段を講じ，ISRにて定量値の再現性を評価することは必要である．

《重要試薬》

Q25. 重要試薬の有効期限の設定は必要か？

A25. 使用する期間中の検量線やQC試料の分析結果の評価から，重要試薬の品質を確認できるならば，有効期限の設定は必ずしも必要でない．

事　務　連　絡
平成 26 年 5 月 30 日

各都道府県薬務主管課　御中

厚生労働省医薬食品局審査管理課

「医薬品開発における生体試料中薬物濃度分析法のバリデーションに関するガイドライン（リガンド結合法）」等の英文版の送付について

標記について、別添１及び２のとおり取りまとめましたので、貴管下関係業者に対して周知方お願いします。

別添１　Guideline on Bioanalytical Method（Ligand Binding Assay）Validation in Pharmaceutical Development

別添2　Questions and Answers (Q&A) for the Guideline on Bioanalytical Method（Ligand Binding Assay）Validation in Pharmaceutical Development

別添 1

Guideline on Bioanalytical Method (Ligand Binding Assay) Validation in Pharmaceutical Development

Table of Contents

1. Introduction

In the development of medicinal products, bioanalytical methods are used in clinical and non-clinical pharmacokinetic studies (including toxicokinetic studies) to evaluate the efficacy and safety of drugs and their metabolites. Drug concentrations determined in biological samples are used for the assessment of characteristics such as *in vivo* pharmacokinetics (absorption, distribution, metabolism, and excretion), bioavailability, bioequivalence, and drug-drug interaction.

It is important that these bioanalytical methods are well characterized throughout the analytical procedures to establish their validity and reliability.

This guideline serves as a general recommendation for the validation of ligand binding assays (LBAs) as bioanalytical methods to ensure adequate reliability. It also provides a framework for analysis of study samples by using validated methods to generate study results supporting applications for drug marketing authorization.

Flexible adjustment and modification can be applied in the case of specialized analytical method or depending on the intended use of the result of analysis. Adjustments and modifications may include appropriate predefinition of acceptance criteria based on scientific rationale.

2. Scope

This guideline is applicable to the validation of LBAs as analytical methods for the measurement of drugs in biological samples obtained in toxicokinetic studies and clinical trials, as well as to the analysis of study samples using such methods. The information in this guideline generally applies to the quantification of peptides and proteins as well as low-molecular-weight drugs that are analyzed by LBAs. A typical example of an LBA is an immunological assay based on antigen-antibody reaction, such as enzyme immunoassay (EIA).

This guideline is not mandatory for analytical methods used in non-clinical studies that are beyond the scope of "Ministerial Ordinance Concerning the Standards for the Conduct of Non-clinical Studies on the Safety of Drugs (Ministry of Health and Welfare ordinance No. 21, dated March 26, 1997)," but could be used as a reference for conducting the required validation of such methods.

3. Reference Standard

A reference standard serves as the scale in quantifying an analyte, and is mainly used to prepare calibration standards and quality control (QC) samples, which are relevant blank matrix spiked with a known concentration of the analyte of interest. The quality of the reference standard is critical, as the quality affect measurement data. A certificate of analysis or an alternative statement that provides information on lot number, content (amount, purity, or potency) and storage conditions should accompany the standard. Also, the expiration date or its equivalent is preferably clarified. As for a reference standard, it is important that the material is procured from an authenticated source and is of well-controlled quality.

4. Analytical Method Validation

An analytical method validation should be performed at every relevant facility when establishing a bioanalytical method for quantification of a drug or its metabolite(s).

4.1. Full validation

A full validation should be performed when establishing a new bioanalytical method for quantification of an analyte/analytes. A full validation is also required when implementing an analytical method that is disclosed in literature or commercialized as a kit product.

The objective of a full validation is to demonstrate the assay performance of the method, e.g., specificity, selectivity, calibration curve, accuracy, precision, dilutional linearity, and stability. Generally, a full validation should be performed for each species or matrix (mainly plasma or serum) to be analyzed.

The matrix used in analytical validation should be as close as possible to the target study samples, including anticoagulants and additives. When an analytical method is to be established for a matrix of limited availability (rare matrix, e.g., tissue, cerebrospinal fluid, bile) and a sufficient amount of matrix cannot be obtained from sufficient number of sources (subjects or animals), a surrogate matrix may be used to prepare calibration standards and QC samples. However, the use of a surrogate matrix should be justified as much as possible in the course of establishing the analytical method.

In an LBA full validation, the minimum required dilution (MRD) should be defined *a priori* (i.e., in the course of method development) to dilute samples with buffer solution.

When using a plate-based LBA, analysis should generally be performed in at least 2 wells per sample; a sample concentration should then be determined either by calculating a mean of responses from the wells or by averaging the concentrations calculated from each response.

4.1.1. Specificity

Specificity is the ability of an analytical method to detect and differentiate the analyte from other substances, including its related substances (i.e., substances that are structurally similar to the analyte). For an LBA, it is important that the binding reagent specifically binds to the target analyte but does not cross-react with coexisting related substances. If presence of related substances is anticipated in biological samples of interest, the extent of the impact of such substances should be evaluated. Specificity may be evaluated in the course of method development. In some cases, an additional specificity testing may have to be conducted after a method validation is completed.

Specificity is evaluated using blank samples (matrix samples without analyte addition) and blank samples spiked with the related substance at concentration(s) anticipated in study samples; in addition, QC samples with the analyte concentrations near the lower limit of quantification (LLOQ) and near the upper limit of the quantification (ULOQ) of calibration curve should be evaluated after spiking with the related substance at anticipated concentration(s).

Assay results for the "neat" blank sample and blank samples spiked with the related substance should be below the LLOQ; and accuracy in the measurements of the QC samples spiked with the related substance should demonstrate an accuracy of within ±20% of the theoretical concentration (or within ±25% of the theoretical concentration at the LLOQ and ULOQ).

4.1.2. Selectivity

Selectivity is the ability of an analytical method to detect and differentiate the analyte in the presence of other components in the samples.

Selectivity is evaluated using blank samples obtained from at least 10 individual sources and near-LLOQ QC samples (i.e., QC samples at or near the LLOQ) prepared using the individual blank samples. In the case of a matrix with limited availability, it may be acceptable to use matrix samples obtained from less than 10 sources.

Assay results for at least 80% of the blank samples should be below the LLOQ; at least 80% of the near-LLOQ QC samples should demonstrate an accuracy of within ±20% of the theoretical concentration (or within ±25% at the LLOQ).

4.1.3. Calibration curve

The calibration curve demonstrates the relationship between a theoretical analyte concentration and its resulting response variable.

A calibration curve should be prepared by using the same matrix as the intended study samples, whenever possible, by spiking the blank matrix with known concentrations of the analyte. A calibration curve should be generated with at least 6 concentration levels of calibration standards, including LLOQ and ULOQ samples, and a blank sample. Anchor point samples at concentrations below the LLOQ and above ULOQ of the calibration curve may also be used to improve curve fitting. A 4- or 5-parameter logistic model is generally used for the regression equation of a calibration curve. The validation report should include the regression equation and weighting conditions used.

The accuracy of back-calculated concentration of each calibration standard should be within ±25% deviation of the theoretical concentration at the LLOQ and ULOQ, and within ±20% deviation at all other levels. At least 75% of the calibration standards excluding anchor points, and a minimum of 6 levels of calibration standards, including the LLOQ and ULOQ, should meet the above criteria.

4.1.4. Accuracy and precision

Accuracy of an analytical method describes the degree of closeness between the analyte concentration determined by the method and its theoretical concentration. Precision of an analytical method describes variation between individual concentrations determined in repeated measurements.

Accuracy and precision are assessed by performing analysis with QC samples, i.e., samples spiked with known amounts of the analyte. In the validation, QC samples with a minimum of 5 different concentrations (LLOQ, low-, mid-, high-levels, and ULOQ) within the calibration range are prepared. The low-level should be within 3 times the LLOQ, the mid-level is near the midpoint on the calibration curve, and the high-level should be at least one-third of the ULOQ of the calibration curve. Accuracy and precision should be evaluated by repeating the analysis in at least 6 analytical runs.

The mean within-run and between-run accuracy at each concentration level should be within ±20% deviation of the theoretical concentration, except at the LLOQ and ULOQ, where it should be within ±25%. Within-run and between-run precision of concentrations determined at each level should not exceed 20%, except at the LLOQ and ULOQ, where it should not exceed 25%. Furthermore, a total error (sum of the absolute value of the relative error [i.e., accuracy minus 100%] and precision) at each level should not exceed 30%, except at the LLOQ and ULOQ, where it should not exceed 40%.

4.1.5. Dilutional linearity

Dilutional linearity is assessed to confirm the following: (i) the method can appropriately analyze samples at concentrations exceeding the ULOQ of a calibration curve without influence of a hook effect or prozone; (ii) measured concentrations are not affected by dilution within the calibration range. Dilutional linearity is evaluated by analyzing a QC sample exceeding the ULOQ of a calibration curve and its serial dilutions at multiple concentrations. The absence or presence of response reduction (hook effect or prozone) is checked in the analyzed samples, and if discovered, measures should be taken to eliminate response reduction in study sample analysis. Accuracy and precision in the measurements corrected for the dilution factor should be within ±20% deviation of the theoretical concentration and not more than 20%, respectively.

4.1.6. Stability

Analyte stability should be evaluated to ensure that the concentration is not affected through each step of the process from the sample collection to the analysis. The stability of the anlayte should be assessed under conditions that are as close as possible to the actual circumstances, e.g. sample storage and sample analysis. Careful consideration should be given to the solvent or matrix type, container materials, and storage conditions used in the stability-determination process.

Validation studies should determine analyte stability after freeze and thaw cycles, and after short-term (e.g., at room temperature, on ice, or under refrigeration) and long-term storage. All stability experiments should be performed on samples that have been stored for a time that is longer than the actual storage period.

Stability of the analyte in the stock and working solutions is evaluated using solutions at or near the highest and lowest concentration levels for the actual solution storage

situation. Stability of the analyte in the studied matrix is evaluated using low- and high-level QC samples. The QC samples should be prepared using a matrix that is as close as possible to the actual study samples, including anticoagulant and additives. Stability is evaluated by analysis of at least 3 replicates per QC concentration level before and after stability storage. The mean accuracy in the measurements at each level should be within ±20% deviation of the theoretical concentration, in principle. Other criteria could be used if they are deemed scientifically more appropriate for the evaluation of a specific analyte.

4.2. Partial validation

Partial validation may be performed when minor changes are made to an analytical method that has already been fully validated. The items in a partial validation are determined according to the extent and nature of the changes made to the method.

Typical bioanalytical method changes subjected to a partial validation are as follows: analytical method transfers between laboratories, changes in analytical instruments, changes of the critical reagent lot, changes in calibration range, changes in the MRD, changes in anticoagulant, changes in analytical conditions, changes in sample storage conditions, confirmation of impact by concomitant drugs, and use of rare matrices.

Acceptance criteria used in partial validation should, in principle, be the same as those employed in the full validation.

4.3. Cross validation

Cross validation is primarily conducted when data are generated in multiple laboratories within a study or when comparing analytical methods used in different studies, after a full or partial validation. The same set of QC samples spiked with the analyte or the same set of study samples is analyzed, and the mean accuracy at each concentration level of QC samples or the assay variability in the measurements of study samples is evaluated.

In the cross validation among 2 or more laboratories within a study, the mean accuracy of QC samples (low-, mid-, and high-levels) evaluated by at least 3 replicates at each level, should be within ±30% deviation of the theoretical concentration, in principle, considering intra- and inter-laboratory precision. When using a set of study samples, the assay variability should be within ±30% for at least two-thirds of the samples.

When conducting cross validation between different analytical methods based on different assay principles, both validation procedure and acceptance criteria (i.e., mean accuracy or assay variability) should be separately defined based on scientific judgment according to the type of the analytical methods.

5. Analysis of Study Samples

Study samples are biological specimens that are obtained from toxicokinetic studies and clinical trials for analysis. Analysis of study samples should be carried out using a fully validated analytical method. During analysis, study samples should be handled under conditions that are validated for adequate stability, and analyzed within a confirmed stability period, along with a blank sample, calibration standards at a minimum of 6 concentration levels, and QC samples at a minimum of 3 concentration levels. In a plate-based LBA, assay should generally be performed in at least 2 wells per sample prepared. A sample concentration should then be determined either by calculating a concentration from an average of each response or by averaging the concentrations calculated from each response.

Validity of the analytical method during study sample analysis should be evaluated in each analytical run by using the calibration curve and QC samples. In a plate-based assay, each plate represents a single analytical run. In studies that serve pharmacokinetic data as a primary endpoint, reproducibility of the analytical method should be confirmed for each representative study per matrix by performing incurred sample reanalysis (ISR: reanalysis of incurred samples in a separate analytical run on a different day to determine whether the original analytical results are reproducible).

5.1. Calibration curve

A calibration curve is used to determine the concentration of the analyte of interest in study samples. A calibration curve used in study sample analysis should be generated for each analytical run by using the validated analytical method. The same model as in the bioanalytical method validation should be used for the regression equation and weighting conditions of the calibration curve.

The accuracy of back-calculated concentrations of calibration standards at each level should be within ±25% deviation of the theoretical concentration at the LLOQ and ULOQ of the calibration curve, and ±20% deviation at all other levels. At least 75% of

the calibration standards excluding anchor points, with a minimum of 6 levels, should meet the above criteria.

If the calibration standard at the LLOQ or ULOQ does not meet the criteria in study sample analysis, the next lowest/highest-level calibration standard may be used as the LLOQ or ULOQ of the calibration curve. Even though narrowed, the modified calibration range should still cover at least 3 different QC sample levels (low-, mid-, and high-levels).

5.2. QC samples

QC samples are analyzed to assess the validity of the analytical method used for calibration curve and study sample analysis.

QC samples with a minimum of 3 different concentration levels (low-, mid-, and high-levels) within the calibration range are analyzed in each analytical run. Usually, the low-level is within 3 times the LLOQ, the mid-level is in the midrange of the calibration curve, and the high-level needs to be at least one-third of the ULOQ of the calibration curve. QC samples are processed in the same manner as with study samples. The analysis requires 2 QC samples at each QC level or at least 5% of the total number of study samples in the analytical run, whichever is the greater.

The accuracy of measurement of QC samples should be within ±20% deviation of the theoretical concentrations. At least two-thirds of the QC samples and at least 50% at each concentration level should meet the above criterion.

5.3. ISR (Incurred sample reanalysis)

In bioanalysis, it can happen that the results of analyses of study samples are not reproducible, even when the method validation is successfully conducted and the validity of at each analytical run is confirmed by calibration standards and QC samples. Such failures can be attributed to various factors, including inhomogeneity of study samples, contamination and other operational errors, and interference of biological components unique to the study samples or of unknown metabolites. ISR refers to reanalysis of incurred samples in separate analytical runs on different days to check whether the original analytical results are reproducible. Confirmation of the reproducibility by ISR improves the reliability of the analytical data. In addition, a failure to demonstrate the reproducibility of the original data in ISR can trigger a cause investigation and remedial

measures for the analytical method.

Usually, ISR is performed for representative studies selected for each matrix in studies that use pharmacokinetic data as the primary endpoint. For instance, ISR should be conducted in the following situations: non-clinical toxicokinetic studies for each different species, representative clinical pharmacokinetic studies in healthy volunteers and patients with renal/hepatic impairment, as well as bioequivalence studies. For non-clinical studies, ISR may be performed with samples obtained in a independent non-GLP study, if the study design is similar to the relevant toxicokinetic study in terms of sampling conditions.

ISR should be performed with samples from as many subjects or animals as possible, including those near the maximum blood concentration (C_{max}) and the elimination phase, within a time window that ensures the analyte stability. As a guide, approximately 10% of the samples should be reanalyzed in cases where the total number of study samples is less than 1000 and approximately 5% of the number of samples exceeding 1000.

The results of ISR are evaluated using assay variability. Assay variability can be calculated as the difference between the concentration obtained by ISR and that in the original analysis divided by their mean and multiplied by 100. The assay variability should be within ±30% for at least two-thirds of the samples analyzed in ISR. In case the ISR data failed to meet the above criteria, root cause investigation should be conducted for the analytical method and necessary measures should be taken by considering the potential impact on study sample analysis.

It should be noted that ISR is performed to monitor assay variability. The original data should never be discarded or replaced with the reanalysis data even if the assay variability exceeds ±30% in a specific sample.

6. Points to note

6.1. Calibration range

In LBAs, calibration range is largely dependent on the characteristics of the binding reagent and it may be difficult to arbitrarily determine the range. In addition, because the calibration range of LBA is comparably narrow, dilutional linearity should be appropriately established to bring the concentrations of analyte in diluted study samples within the range of the calibration curve.

In case the calibration range is changed, partial validation should be performed.

However, it is not necessary to reanalyze the study samples that have been quantified prior to the change in the calibration range.

6.2. Reanalysis

Possible reasons and procedures for reanalysis, as well as criteria for handling of concentration data should be defined *a priori* in the protocol or standard operating procedure (SOP).

Examples of reasons for reanalysis are as follows: calibration curve or QC samples failed to meet the criteria for validity of the analytical run; the obtained concentration exceeded the ULOQ of the calibration curve or fell below the LLOQ due to excess dilution; the analyte of interest was detected in pre-dose or placebo samples; improper analytical operation or malfunction of analytical instrument; and causal investigation on abnormal values.

Reanalysis of study samples for pharmacokinetic reasons should be avoided, whenever possible. Particularly in bioequivalence studies, it is not acceptable to reanalyze study samples and modify the concentration data only because the initial data were pharmacokinetically questionable. However, reanalysis of specific study samples is acceptable when, for instance, the initial analysis yielded an unexpected or anomalous result that may affect the safety of subject in a clinical trial.

In any case, when reanalysis is performed, the analytical report should provide information of the reanalyzed samples, the reason for reanalysis, the data obtained in the initial analysis, if any, the data obtained in the reanalysis, and the final accepted values and the reason and method of selection.

6.3. Carry-over

Carry-over is an alteration of a measured concentration due to residual analyte in the analytical equipment.

Carry-over is not an issue for analyses performed in plates and tubes, while it should be taken into account in analyses that use a single flow cell, flow path, and/or autosampler.

If carry-over is inevitable, its impact needs to be examined, and appropriate measures should be taken to avoid any impact on the actual study sample analysis. Should there be any concern that carry-over may affect the quantification of analyte in study samples, it

should be evaluated during the actual study sample analysis to assess the impact on the concentration data.

6.4. Cross-talk

Cross-talk is an alteration of a measured concentration due to a leak of fluorescence or luminescence to adjacent wells in plate-based assay.

If cross-talk is inevitable, its impact needs to be examined, and appropriate measures should be taken to avoid any impact on the actual study sample analysis. Should there be any concern that cross-talk may affect the quantification of analyte in study samples, this should be evaluated during the actual study sample analysis to assess the impact on the concentration data.

6.5. Critical reagents

A critical reagent is the one that has a direct impact on the results of an LBA-based bioanalytical method and usually includes, but is not limited to, binding reagents (e.g., unlabeled or labeled antibodies).

A critical reagent should be selected by considering the specificity for the analyte and should be stored under conditions that ensure consistent quality. The quality of critical reagent should be appropriately maintained throughout the period of use in analytical method validation and study sample analysis. Partial validation is in principle required when the critical reagent lot is changed.

6.6. Interfering substances

Interfering substances are those that may affect the concentration data in study sample analysis and may include, but are not limited to, soluble ligands and anti-drug antibodies.

If interfering substances are potentially present in study samples, it is advisable to examine the impact of interfering substances on the concentration data.

7. Documentation and Archives

In order to ensure adequate reproducibility and reliability of bioanalysis, results obtained in analytical method validations and study sample analyses should be

documented in a validation report and a study sample analysis report as described below. The reports should be stored along with relevant records and raw data in an appropriate manner.

All relevant records and raw data should be kept, including those obtained in rejected analytical runs, specifically record of reference materials, blank matrices, and critical reagents (receipt/release, use, and storage), record of samples (receipt/release, preparation, and storage), record of analyses, record of instrument (calibration and settings), record of deviations, record of communications, and raw data such as analytical data.

Validation report

- Summary of the validation
- Information on the reference standards
- Information on the blank matrices
- Information on the critical reagents
- Analytical method (including description related to the MRD)
- Validated parameters and the acceptance criteria
- Validation results and discussion
- Rejected runs together with the reason for rejection
- Information on reanalysis
- Deviations from the protocol and/or SOP, along with the impact on study results
- Information on reference study, protocol, and literature

Study sample analysis report

- Summary of the study sample analysis
- Information on the reference standards
- Information on the blank matrices
- Information on receipt and storage of study samples
- Information on the critical reagents
- Analytical method (including description related to the MRD)
- Parameters, acceptance criteria, and results of the validity evaluation
- Results and discussion of study sample analysis

- Rejected runs together with the reason for rejection
- Information on reanalysis
- Deviations from the protocol and/or SOP, along with impact on study results
- Information on reference study, protocol, and literature