EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Submission of comments on ICH guideline M10 on bioanalytical method validation

(EMA/CHMP/ICH/172948/2019)

Comments from:

Name of organisation or individual
This document contains the consolidated comments from the European Bioanalysis Forum vzw (EBF vzw, non-profit). Following companies (Pharma R&D and CRO) are member of the EBF vzw
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Please note that comments will be sent to the relevant **ICH EWG** for consideration in the context of Step 3 of the ICH process.

1. General comments

Stakeholder number *(To be completed by the Agency)*	General comment (if any)
	Overall comment from the European Bioanalysis Forum (EBF) community is that the document is well written. Nevertheless, the EBF has suggestions to ensure that the final ICH M10 is not only a harmonised guideline for BMV, but also supports method development, validation/qualification and study sample analysis for all phases of pharmaceutical R&D in the most resource effective way (from ICH mission: ICH's mission is to achieve greater harmonisation worldwide to ensure that safe, effective, and high quality medicines are developed and registered in the most resource-efficient manner. *Ref: https://www.ich.org/about/mission.html).* The comments in this document are the consolidated opinion from the EBF member companies mentioned above and are very similar to the EFPIA comments which were provided as a separated file via EFPIA. The background is that virtually all EFPIA member companies are also a member of the EBF and the comments were gathered through identical surveys amongst the EBF and EFPIA members, from discussions as part of EBF and AAPS workshops on ICH M10, held in Barcelona (May 2019) and Silver Spring (June 2019) respectively. A few comments were given specifically by the EBF, which are added in this document. In order to facilitate triage, they are highlighted with red line numbers in the column "line N°". For a few comments, the EBF refined the view or comment as given by EFPIA. In those cases, the line numbers are highlighted as 'strike through' (e.g. ~~714 722~~) and should not be considered. All other comments with line numbers in black are identical to the comments given by EFPIA General remarks (refinements as proposed in our comments further in the document are of significant importance for industry): • "Scope" is generally perceived as too broad and ambiguous. If unchanged, all studies, all matrices and all analytes are at risk of becoming in scope. • Some parts of stability assessment are perceived as too broad. Example given is co-med stability assessment.

Stakeholder number *(To be completed by the Agency)*	General comment (if any)
	• Consider harmonised decision-based acceptance criteria rather than technology-based ones (LCMS vs LBA). (*Ref: Bioanalysis (2018) 10(16), 1255–1259*). Also, this would prepare the Guideline for future technologies entering the regulatory BMV space. • "Table 1: Documentation" and "Paragraph 2.1: Method Development" carry the risk of becoming overinterpreted and are increasing the resource requirements for industry, whilst stifling scientific freedom required in the method development arena (and not aligned with the mission of ICH). ➢ For "Documentation" we suggest to limit the requirements in table 1 to BA/BE-studies, and allow reporting of other studies to be less detailed (i.e. less in reports but allow documentation to be available at the analytical site) ➢ For "Method Development," we suggest to limit to scope to changes to already validated methods in later stages of development. • 3Rs: EFPIA feels that a sustainable and science based guideline should consider animal welfare and not require unnecessary use of animals. (*Ref: https://www.nc3rs.org.uk/the-3rs*) ➢ Replace = allow surrogate matrix used when proven valid (e.g. sample dilutions, calibrators,..) ➢ Reduce = using smaller volumes/less replicates of sample or matrix in preclinical assays ➢ Refine = facilitate micro-sampling assays More details on above general comments are provided below. Suggested changes are given in red. Where available, the justification is provided in blue .

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes
176-180		Comment: refine Proposed change (if any): This guideline is intended to provide recommendations for the validation of bioanalytical methods for chemical and biological drug quantification and their application in the analysis of study samples. Adherence to the principles presented in this guideline will ensure the quality and consistency of the bioanalytical data in support of the development and market approval of both chemical and biological drugs.
181-185		Comment: refine Proposed change (if any): The objective of the validation of a bioanalytical method is to demonstrate that it is appropriate for its intended purpose. Changes from the recommendations in this guideline may be acceptable if appropriate scientific justification is documented and provided upon request to regulatory authorities. Applicants are encouraged to consult the regulatory authority(ies) regarding significant changes in method validation approaches when an alternate approach is proposed or taken
187-193		Comment: add - (proposal deconvolutes background from scope) Proposed change (if any): Concentration measurements of chemical and biological drug(s) and their metabolite(s) in biological matrices are an important aspect of drug development. The results of studies employing such methods contribute to regulatory decisions regarding the safety and efficacy of drug products. It is therefore critical that the bioanalytical methods used are well characterised, appropriately validated and documented in order to ensure reliable data to support regulatory decisions.
195-204		Comment: Suggested changes of or additions to the paragraph in red Proposed change (if any): This guideline describes the method validation that is expected for bioanalytical assays that are submitted to support regulatory submissions. The guideline is applicable to the validation of bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma,

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		serum, other body fluids ~~or tissues~~) obtained in nonclinical TK studies falling under the scope of the GLPs that are used to make regulatory decisions, nonclinical PK studies that are conducted as surrogates for clinical studies, and all phases of clinical trials in regulatory submissions for which a primary objective of the study is to assess, compare or characterize drug exposure. Full method validation is expected for the primary matrix(ces) intended to support regulatory submissions. Primary matrix(ces) are identified based on the objective(s) of individual studies and these should be indicated in the study protocol or sample analysis plan. For non-primary analytes/matrices validation should be performed in line with the anticipated use of the data, using the appropriate/applicable principles (i.e. partial validation or alternative approaches). ~~The analytes that should be measured in nonclinical and clinical studies and the types of studies necessary to support a regulatory submission are described in other ICH and regional regulatory documents.~~ *(proposal to delete last sentence as it creates more confusion than clarity and opens ICH M10 to become dependent on (future) regional regulations)*
205-207		Comment: delete - *(one does not know a priori if a study will be considered for regulatory decisions, thus in practice, only limiting to studies not included in submissions is possible)* Proposed change (if any): For studies that are not submitted for regulatory approval ~~or not considered for regulatory decisions regarding safety, efficacy or labelling (e.g., exploratory investigations)~~, applicants may decide on the level of qualification that supports their own internal decision making.
212-213		Comment: rephrase Proposed change (if any): For studies that are subject to Good Laboratory Practice (GLP) the bioanalysis of study samples must also conform to its requirements. In accordance with Good Clinical Practice (GCP), the bioanalysis of clinical study samples must be conducted as described by the study protocol and within the limits of the informed consent agreed to by study participants
217		Comment: General comment – if comment to delete the section is not considered, more editorial comments are provided from line 224 onwards, albeit this would be our second choice. As first suggestion, we don't provide alterative text since the proposal is to delete this section: The method development is a

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		previous work that only concerns the laboratories and their organization and knowhow to obtain robust, accurate and precise assays. Nevertheless, once fully validated, it is considered relevant to provide information on the method changes, the evolution of methods and reasons, as it is commented in a very correct way is section 8.1 for the CTD Proposed change (if any): delete this section
224-226		Comment: There should not be an expectation that all parameters are fine tuned to an **optimum**. Proposed change (if any):
224-226		Comment: Recommendation to rephrase verbiage around method development (MD) activities such as "MD can/may involve assessments of the following" or delete list as it becomes a risk of being a mandated requirement Proposed change (if any): delete bullets
238-240		Comment: add Proposed change (if any): However, the applicant should record the changes to procedures as well as any issues and their resolutions to provide a rationale for any changes made to validated methods (i.e. Method Evolution) immediately prior to or in the course of analysing study samples for pivotal studies.
238-240		Comment: add Proposed change (if any): Formal reporting is not required, however a proper use of the CTD detailing rationale for any changes is encouraged.
241-242		Comment: Rephrase

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): Once the method has been developed, the method is ready for validation to generate a validated method which is suited for sample analysis
262-266		Comment: Suggest specifying that a change in counterion is considered the same anticoagulant. We suggest editing the text to read: Proposed change (if any): The matrix used for analytical method validation should be the same as the matrix of the study samples, including anticoagulants and additives. A different counterion is considered the same anticoagulant (e.g., Na/Li-heparin; K_2/K_3 EDTA). In some cases where rare matrices are considered primary matrix, it may be difficult to obtain an identical matrix to that of the study samples. In such cases, surrogate matrices may be acceptable for analytical method validation. The alternative matrix should be selected and justified scientifically for use in the analytical method.
276-279		Comment: Propose to use identical text as 6.2 to prevent confusion: Proposed change (if any): Cross validation is required (i) when data are obtained from different fully validated methods within a study, (ii) data are obtained from different fully validated methods across studies that are going to be combined or compared to support special dosing regimens, or regulatory decisions regarding safety, efficacy and labelling, or (iii) Data are obtained within a study from different laboratories with the same bioanalytical method. (Refer to Section 6.2)
281		Comment: general comment: Acknowledge the molecular diversity (which includes peptide and proteins) in chromatography and the impact on reference standards. Current section 3.1 is written around NCE. Proposed change (if any):
286-288		Comment: delete, The validation data justifies the absence of the IS. Proposed change (if any): ~~The absence of an IS should be technically justified~~

Line no.	Stakeholder no.	Comment and rationale; proposed changes
286-288		Comment: rephrase Proposed change (if any): It is recommended to add a suitable internal standard (IS) ~~should be added~~ to all calibration standards, QCs and study samples during sample processing.
292-294		Comment: delete Proposed change (if any): ….study sample analysis should be obtained from an authentic and traceable source. ~~The reference standard should be identical to the analyte. If this is not possible, an established form (e.g., salt or hydrate) of known quality may be used.~~
295-296		Comment: delete Proposed change (if any): Suitable reference standards include compendial standards, commercially available standards or sufficiently characterised standards prepared in-house or by an external ~~non-commercial~~ organisation.
297-299		Comment: add Proposed change (if any): A certificate of analysis (CoA) or an equivalent alternative is required to ensure quality and to provide information on the purity, storage conditions, retest or expiration date, batch or lot number and manufacturer or source of the reference standard
304-306		Comment: add Proposed change (if any): The presence of unlabelled analyte should be checked and if unlabelled analyte is detected, the potential influence should be evaluated and/or reduced to an acceptable level during method validation.
307-309		Comment: delete

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): Stock and working solutions can only be prepared from reference standards that are within the stability period as documented in the CoA (either expiration date or the retest date) ~~in early development phase).~~
314-317		Comment: Suggested acceptance criteria: add Proposed change (if any): Selectivity is evaluated using blank samples (matrix samples processed without addition of an analyte or IS) obtained from at least 6 individual sources/lots (non-haemolysed and non-lipaemic). At least 5 out of 6 should pass. Use of fewer sources may be acceptable in the case of rare matrices. Selectivity for the IS should also be evaluated.
314-317		Comment: The number of individual matrix sources required for selectivity and matrix effect assessment should take into account the diversity of the study population. An assessment in a single lot may be satisfactory for a study in a non-diverse nonclinical population - add Proposed change (if any): Selectivity is evaluated using blank samples (matrix samples processed without addition of an analyte or IS) obtained from at least 6 individual sources/lots (non-haemolysed and non-lipaemic). Use of fewer sources may be acceptable in the case of rare matrices and when scientifically justified for non-clinical matrices. Selectivity for the IS should also be evaluated.
323-324		Comment: Instead of routine, this should be included as a for cause experiment: Proposed change (if any): If required, ~~For the~~ the investigation of selectivity in lipaemic matrices at least one source of matrix should be used
332-334		Comment: rephrase Proposed change (if any): If required ~~For the~~ investigation of selectivity in haemolysed matrices at least one source of matrix should be used. Haemolysed matrices are obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) ~~to generate~~

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		~~a visibly detectable haemolysed sample.~~
343-345		Comment: rephrase Proposed change (if any): In the case of ~~LC~~-MS based methods, to assess the impact of such substances, the evaluation may ~~include~~ be done by comparing the molecular weight…
355-356		Comment: rephrase Proposed change (if any): The extent of back-conversion should be established, controlled where possible. If present, the impact on the study results should be discussed in the Bioanalytical Report.
357		Comment: 2 levels x 3 replicates x 6 sources (+ haemolysed + lipaemic) = excessive for ALL validations at all stages of development… also, consider 3Rs (purpose bred – 1 per species should be sufficient). Proposed change (if any):
366-370		Comment: refine Proposed change (if any): The matrix effect should also be evaluated in haemolysed and hyperlipidaemic control matrix, where applicable, and in relevant patient populations or special populations (e.g., hepatically impaired or renally impaired) when available. QC samples should be prepared in at least a single source/lot of this control matrix, at LOW and HIGH concentrations and should be extracted and analysed. The acceptance criteria (RE and CV) is the same as for the assessment of intra-batch accuracy and precision. The evaluation of lipaemic matrices is not necessary for preclinical studies unless the drug impacts lipid metabolism or is administered in a particular animal strain that is hyperlipidaemic'
375-378		Comment: add

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): ….Calibration standards should be prepared in the same biological matrix as the study samples. (delete: The calibration range is defined by the LLOQ, which is the lowest calibration standard, and the ULOQ, which is the highest calibration standard.). For rare and difficult to obtain matrices, tissues and endogenous methods, the use an appropriate surrogate matrix is allowed. The calibration range should be appropriate for the analysis of samples. In the event that a significant number of samples require diluting into the calibration range, partial validation of an overlapping or non-overlapping higher calibration range is recommended. There should be one calibration curve for each analyte studied during method validation and for each analytical run
379-381		Comment: rephrase Proposed change (if any): A calibration curve should be generated with ~~a blank sample, a zero sample (blank sample spiked with IS), and~~ at least 6 concentration levels of calibration standards, including the LLOQ and the ULOQ. A blank sample and zero sample should be included in the run.
379-381		Comment: add Proposed change (if any): Additional concentration levels are required when non-linear e.g. quadratic, regression analysis is used.
382-388		Comment: Regression model selection needs to be documented (SOP) isn't needed. Suggest to remove. Proposed change (if any):
397-404		Comment: There is a clear discrepancy in criteria when a single CAL or replicates are used. When a single CAL is used, it is accepted to fail in one conc. level if at least 6 con. levels remain. However, when 2 CALs/level are used, and two CALs/level fail (not the 50%, as stated), but there are also 6 levels remaining, the calibration curve is not accepted. (this is also in contradiction with the section "3.3.2 Acceptance Criteria for an Analytical Run" when it is stated that "If replicate calibration standards are used and only one of the LLOQ or ULOQ standards fails, the calibration range is unchanged".

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): delete: ~~In the case that replicates are used, the criteria (within ±15% or ±20% for LLOQ) should also be fulfilled for at least 50% of the calibration standards tested per concentration level.~~
403-404		Comment: rephrase Proposed change (if any): delete: … should be rejected, the possible source of the failure should be determined ~~and the method revised if necessary. If the next validation run also fails, then the method should be revised before restarting validation.~~
419-421		Comment: change Proposed change (if any): During method validation the QCs should be prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within three times of the LLOQ (low QC), around geometric mean of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC).
436-437		Comment: remove "(intermediate)" Proposed change (if any): Between-run ~~(intermediate)~~ precision and accuracy should be calculated by combining the data from all runs.
458-461		Comment: in consideration of 3Rs, delete Proposed change (if any): Dilution integrity …… The same matrix ~~from the same species~~ used for preparation of ……..should not exceed 15%.
462-468		Comment: It is difficult to determine in validation which exact dilution ratio will be needed during sample analysis. Currently, one dilution ratio is performed during validation. At the time of sample analysis, QCs prepared above the ULOQ are diluted at the same ratio as study samples and included in the run which is a sufficient evaluation of dilution integrity. Delete

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): ~~The dilution ratio(s) applied during study sample analysis should be within the range of the dilution rations evaluated during validation. The mean accuracy of the dilution QCs should be within ±15% of the nominal concentration and the precision (%CV) should not exceed 15%.~~
469-470		Comment: rephrase Proposed change (if any): ~~In~~ The ~~cases of rare matrices~~ use of a surrogate matrix for dilution may be acceptable, as long as it has been demonstrated that this does not affect precision and accuracy
480-483		Comment: industry remains unclear on the requirements to evaluate in triplicate/QC level Proposed change (if any):
480-483		Comment: rephrase Proposed change (if any): Stability of the analyte in the studied matrix is evaluated using low and high concentration stability QCs. Aliquots of the low and high stability QCs are analysed ~~at time zero~~ and after the applied storage conditions that are to be evaluated. Analysis of the stability QCs prior to storage (e.g. at t=0) may be informative with respect to confirming that they have been correctly prepared, but is not required.
489-490		Comment: delete Proposed change (if any): …unless it is recognised that this may not be possible ~~in nonclinical studies~~ due to solubility limitations.
491-493		Comment: rephrase Proposed change (if any): If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific drug regimen) the stability test of an analyte in matrix containing all dosed compounds should be considered. In the case

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		of a fixed combination stability information of the combination dosage form may be considered. In the case of a drug regimen, the known chemistry and stabilities of the individually dosed drugs should be used as a basis for determining whether additional stability studies are needed. DDI studies are not is scope of this requirement.
496-509		Comment: delete Proposed change (if any): 1) Stability of stock and working solutions The stability of the stock and working solutions of the analyte and IS should be determined under the storage conditions used during the analysis of study samples by using the lowest and the highest concentrations of these solutions. ~~They are assessed using the response of the detector. Stability of the stock and working solutions should be tested with an appropriate dilution, taking into consideration the linearity and measuring range of the detector.~~ If the stability …… solution. ~~The routine practice of making stock and working solutions from reference standards solely for extending the expiry date for the use of the reference standard is not acceptable.~~
516-518		Comment: delete Proposed change (if any): …… cycles undergone by the study samples~~, but a minimum of three cycles should be conducted~~
522-524		Comment: delete Proposed change (if any): ~~Low and high stability QCs should be thawed in the same manner as the study samples and kept on the bench~~
528-534		Comment: rephrase Proposed change (if any): 4) Processed sample stability: The stability of processed samples, including the time until completion of analysis (in the autosampler/instrument), should be determined. ~~For example (i) Stability of the processed sample at the storage~~

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		~~conditions to be used during the analysis of study samples (dry extract or in the injection phase) (ii) On-instrument/ autosampler stability of the processed sample at injector or autosampler temperature.~~
535-544		Comment: delete Proposed change (if any): 5) Long-term matrix stability: The long-term stability of the analyte in matrix stored in the freezer should be established. Low and high stability QCs should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples. ~~For chemical drugs,~~ It is considered acceptable to extrapolate the stability at one temperature (e.g., -20°C) to lower temperatures (e.g., -70°C). ~~For biological drugs, it is acceptable to apply a bracketing approach, e.g., in the case that the stability has been demonstrated at -70°C and at -20°C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.~~
546-553		Comment: rephrase Proposed change (if any): ~~1) Whole blood stability:~~ Sample collection integrity: Sufficient attention should be paid to the ~~stability~~ integrity of the analyte in the sampled matrix ~~(blood)~~ directly after collection from subjects and prior to preparation for storage to ensure that the concentrations obtained by the analytical method reflect the concentrations of the analyte in the subject's ~~blood~~ sample at the time of sample collection. Conditions for sample collection should be identified during method development or validation. If the matrix used is plasma ~~or serum~~, the stability of the analyte in blood should be evaluated considered during method development ~~(e.g., using an exploratory method in blood)~~ , and, in the case of molecules that are, based on their structure, potentially unstable, assessed during method validation. The results of such assessments or, in the event they are not conducted, rationale for their absence, should be provided in the Validation Report.
554		Comment: change title Proposed change (if any): 3.2.9. Processed sample Viability

Line no.	Stakeholder no.	Comment and rationale; proposed changes
555-559		Comment: replace Proposed change (if any): The viability of processed samples supporting the storage of processed samples before analysis and re-analysis in the event of an analytical run failing to complete or the entire run needing to be reinjected due to technical error, including the time until completion of analysis (in the autosampler/instrument), should be determined. Re-inject a stored run, comprising of calibration and QC samples, if sufficient processed sample volume permits. The re-injected run should include a minimum n=5 replicates of the low and high QCs. Calculate the QC results from the re-injected calibration curve regression and assess the assay accuracy and precision criteria.
587-590		Comment: It is suggested to eliminate batch vs. run acceptance approach Proposed change (if any):
591-595		Comment: Some information is redundant with section 3.2.6. Consider reducing redundancy Proposed change (if any):
591-595		Comment: delete Proposed change (if any):… samples, injection of blank samples after samples with an expected high concentration) or the validity of the reported concentrations should be justified ~~in the Bioanalytical Report.~~
608-615		Comment: Consider changing acceptance criteria for the new lower limit calibration standard (after the LLOQ was rejected) to ±20% Proposed change (if any):
616-620		Comment: Consider adding a similar statement in the LBA section

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any):
621-626		Comment: Clarify how many replicates of dilution QCs are expected (2?). Proposed change (if any):
627-630		Comment: Clarify that calibration curve for a given analyte can be prepared as part of a cocktail with other analytes when more than one analyte is quantified Proposed change (if any):
631-636		Comment: Consider removing the "(between-run)" in the "overall (between-run) accuracy and precision". Between run is the residual variability between the runs. Overall is the sample population variability. Proposed change (if any):
631-636		Comment: Consider adding similar level of detail to the LBA section Proposed change (if any):
638-641		Comment: The assay quantification range has been validated during assay validation phase. Please consider removing this requirement or clarifying what QC concentration would satisfy the requirement to adequately reflect study samples concentrations. Proposed change (if any):
648-652		Comment: Consider defining "large number of the analyte concentrations..", for example, by providing % of samples. Alternatively, allow for a sample dilution option

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any):
648-652		Comment: Difficult to manage in an early development setting. Requirement should be limited to BA/BE studies Proposed change (if any):
653-655		Comment: Please clarify the need to have 2 QC levels within the range of sample concentrations if method was already validated for a given range of quantitation and sample concentrations are derived from assay calibration curve, not QCs. Proposed change (if any):
656		Comment: Clarify that this includes re-injection of same run and re-extraction of same run. - add in paragraph Proposed change (if any): For study samples involving multiple analytes, a valid result for one analyte should not be rejected because of another analyte failing the acceptance criteria. Additionally if a sample is re-analysed because one of the analytes failed to meet acceptance criteria the data for the analyte(s) that previously met acceptance criteria need not be regressed.
656		Comment: Many comments came in asking for detailed clarification. An overarching theme was to consider clarifying that reanalysis is related to samples that produced valid results (e.g. >ULOQ or <LLOQ). Reanalysis of samples from failed or rejected runs that did not produce acceptable results should not be viewed as "reanalysis" in the context of this paragraph. Hence we suggest to clearly separated the examples to reflect both cases • Reanalysis of a sample which didn't give a reportable concentration, is not reanalyses per se but generates a 1st reportable result • Reanalysis of a sample for which the 1st reportable result is 'unexpected' (positive placebo, unexpected PK,…), is reanalysis. It should be performed in replicate and compared to the original result with the aim to confirm or disprove this

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		original result. An SOP /decision tree should be in place to guide reanalysis and reporting Proposed change (if any):
697-702		Comment: rephrase Proposed change (if any): Chromatogram integration and reintegration should be described in a study plan, protocol or SOP. Any deviation from the procedures described a priori should be discussed in the Bioanalytical Report. Chromatogram integration parameters and in case of re-integration, initial and the final integration data should be documented at the laboratory and should be available upon request
697-702		Comment: The term "re-integration" needs to be defined. For example, consider: "integration that occurs after the initial save of the results table." Proposed change (if any):
706-713		Comment: add Proposed change (if any): …. possible, or from a batch which has shown analytical comparability. If the reference standard batch used for bioanalysis is changed, bioanalytical evaluation should be carried out prior to use to ensure that the performance characteristics of the method are within the acceptance criteria and to ensure consistency of results between batches in case of change during bioanalysis of samples from a given nonclinical or clinical study
715-721		Comment: rephrase Proposed change (if any): ~~Critical reagents bind the analyte and, upon interaction, lead to an instrument signal corresponding to~~

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		~~the analyte concentration.~~ Critical reagents, including binding reagents (e.g. binding proteins, aptamers, antibodies or conjugated antibodies), have direct impact on the results of the assay and therefore their quality must be assured. The critical reagents should be identified and defined in the assay method~~. Reliable procurement of critical reagents, whether manufactured in-house or purchased commercially, should be considered early in method development.~~
725-734		Comment: rephrase – *justification There is ambiguity with respect to minor vs major. What is e.g. the difference between minor (source of reagent is changed) vs major (change in production method, new supplier for antibody). Therefore, the suggestion is to bring one clear example for minor, 1 clear example for major (as proposed above), and leave it a scientific decision to define what is minor vs major.* Proposed change (if any): ….for characterisation, with proper documentation kept at the analytical site. If the change is major (e.g. switch from antigen-based detection molecule to an antibody-based detection molecule), then additional validation experiments are necessary. Ideally, assessment of changes will compare the assay with the new reagents to the assay with the old reagents directly. ~~Major changes include, but are not limited to, change in production method of antibodies, additional blood collection from animals for polyclonal antibodies and new clones or new supplier for monoclonal antibody production.~~
738-740		Comment: add Proposed change (if any): …The performance parameters should be documented at the analytical site in order to support the extension or replacement of the critical reagent.
742-749		Comment: add Proposed change (if any):… the replicate wells or by averaging the concentrations calculated from each response. Acceptance criteria regarding the mean of the response or concentrations values should be predefined. Data evaluation should be performed on reportable concentration values.
750		Comment: consider adding a definition, as e.g. done with Selectivity

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any):
751-752		Comment: Add definition of what consists a 'structurally related molecule' Proposed change (if any):
~~753-757~~		Comment: add/delete Proposed change (if any): The accuracy of the target analyte at the LLOQ and at the ~~ULOQ~~ High QC should be investigated in the presence of related molecules at the maximal concentration(s) anticipated in study samples. The response of blank samples spiked with related molecules should be below the LLOQ. The accuracy of the target analyte in presence of related molecules should be within ±25% and ±20% of the nominal values for the LLOQ and High QC spike respectively.
753-757		Comment: delete – data driven discussions in EBF challenge the scientific need for including the ULOQ Or even a High QC Proposed change (if any): The accuracy of the target analyte at the LLOQ ~~and at the ULOQ~~ should be investigated in the presence of related molecules at the maximal concentration(s) anticipated in study samples. The response of blank samples spiked with related molecules should be below the LLOQ. The accuracy of the target analyte in presence of related molecules should be within ±25% of the nominal values..
758-763		Comment: rephrase Proposed change (if any): In the event of non-specificity, the impact on the method should be evaluated by spiking increasing concentrations of interfering molecules in blank matrix and measuring the accuracy of the target analyte at the LLOQ and ~~ULOQ~~ High QC/at the maximal expected sample concentration of the target analyte. It is essential to …
768-771		Comment: delete

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): Selectivity is the ability of the method to detect and differentiate the analyte of interest in the presence of ~~other~~ "unrelated compounds" (non-specific interference) in the sample matrix.
772-776		Comment: delete Proposed change (if any): … is evaluated using blank samples obtained from at least 10 individual sources and by spiking the individual blank matrices at the LLOQ ~~and at the high QC level~~. The response of the blank samples should be below the LLOQ in at least 80% of the individual sources.
779-782		Comment: Add text from 3.2.1 rather than a reference, including suggested edits to the original draft Proposed change (if any):
779-782		Comment: rephrase Proposed change (if any): Selectivity should be evaluated in lipaemic samples and haemolysed samples if relevant. For lipaemic and haemolysed samples, tests can be evaluated once using a single source of matrix as part of the 10 individuals……In the case of relevant patient populations, when available, there should be at least five individual patients.
784-790		Comment: add Proposed change (if any): …Calibration standards and QCs prepared in a matrix different from the study samples should be justified and appropriate experiments should be performed"
791-797		Comment: consider deleting blank matrix (blank sample), as there is no added value on having this in each and every analytical run → delete

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): A calibration curve should be generated with at least 6 concentration levels of calibration standards, including LLOQ and ULOQ standards~~, plus a blank sample. The blank sample should not be included in the calculation of calibration curve parameters.~~ Anchor point samples at concentrations below the LLOQ and above the ULOQ of the calibration curve may also be used to improve curve fitting. The relationship between response and concentration for a calibration curve is most often fitted by a 4- or 5-parameter logistic model if there are data points near the lower and upper asymptotes, although other models may be used with suitable justification.
798-799		Comment: propose to delete - Justification: suggest deleting this sentence as it might create ambiguity. These factors are considered anyway during A&P runs. In the way it is written now, you can interpret that you have to do these 6 runs, followed by at least A&P runs. Proposed change (if any):
800-805		Comment: precision never can be negative Proposed change (if any): The precision should be within 20%, except for LLOQ and ULOQ where precision should be within 25%.
806-808		Comment: rephrase Proposed change (if any): … If freshly spiked calibration standards are not used, the frozen calibration standards can be used ~~within their defined period of stability~~. under the condition that freeze-thaw stability and a defined period of stability has been proven for the calibrator standards.
811-813		Comment: add Proposed change (if any): Calibration standards and QCs prepared in a matrix different from the study samples should be justified and appropriate experiments should be performed"

Line no.	Stakeholder no.	Comment and rationale; proposed changes
814-819		Comment: delete – justification: the reference standard is usually a single liquid stock Proposed change (if any): The dilution series for the preparation of the QCs should be completely independent from the dilution series for the preparation of calibration standard samples. ~~They may be prepared from a single stock provided that its accuracy has been verified or is known.~~ The QCs should be prepared at a minimum of 5 concentration levels within the calibration curve range: The analyte should be spiked at the LLOQ, within three times of the LLOQ (low QC), around the geometric mean of the calibration curve range (medium QC), and at least at 75% of the ULOQ (high QC) and at the ULOQ
824-831		Comment: rephrase Proposed change (if any):… Within-run accuracy and precision data should be reported for each run, unless there was an obvious documented error to justify the rejection of a run(s). An overall calculation of within-run accuracy and precision for each QC level should be determined. Within-run accuracy or precision criteria do not need to be met in all runs for the assessment to be successful. Between-run precision and accuracy should be calculated by combining the data from all runs.
843-848		Comment: rephrase Proposed change (if any): Due to ~~the narrow assay range~~ high analyte concentrations in study samples in many LBAs, study samples may require dilution in order to achieve analyte concentrations within the range of the assay. Dilution linearity is assessed to confirm: (i) that measured concentrations are not affected by dilution within the calibration range and (ii) the absence or presence of a hook effect. ~~that sample concentrations above the ULOQ of a calibration curve are not impacted by hook effect (i.e., a signal suppression caused by high concentrations of the analyte), whereby yielding an erroneous result.~~ Add: Calibration standards and QCs prepared in a matrix different from the study samples should be justified and appropriate experiments should be performed"
849		Comment: add

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): The same matrix as that of the study sample should be used for preparation of the QCs for dilution. Calibration standards and QCs prepared in a matrix different from the study samples should be justified and appropriate experiments should be performed.
850-856		Comment: add Proposed change (if any): ….with blank matrix (or matrix diluted with buffer) to a concentration within the calibration range. For each dilution ….
850-856		Comment: reason for above additional text (or matrix diluted with buffer) is to prevent erroneous results from diluting with matrix containing endogenous compounds Proposed change (if any):
857-859		Comment: add Proposed change (if any): The calculated concentration for each dilution within the calibration range should be within ±20% of the nominal …..
863		Comment: Include a statement that WBS is not required for LBA assays Proposed change (if any):
866-870		Comment: add Proposed change (if any): The storage and analytical conditions applied to the stability tests, such as the sample storage times (e.g. maximum time between sample collection and sample analysis) and temperatures, ….

Line no.	Stakeholder no.	Comment and rationale; proposed changes
873-874		Comment: rephrase Proposed change (if any): … A minimum of three replicates ~~stability QCs~~ should be prepared and analysed per concentration level/storage condition/timepoint.
880-881		Comment: add Proposed change (if any): …The mean concentration of the three replicates at each level should be within ±20% of the nominal concentration.
882-885		Comment: delete Proposed change (if any):
889-890		Comment: add Proposed change (if any): For both chemical and biological drugs, it is considered acceptable to extrapolate the stability at one temperature (e.g., -20°C) to lower temperatures (e.g., -70°C).
902-907		Comment: delete Proposed change (if any): An analytical run consists of a ~~blank sample~~, calibration standards at a minimum of 6 concentration levels, at least 3 levels of QCs (low, medium and high) applied as two sets (or at least 5% of the number of study samples, whichever is higher) and the study samples to be analysed. ~~The blank sample should not be included in the calculation of calibration curve parameters. The QCs should be placed in the run in such a way that the accuracy and precision of the whole run is ensured taking into account that study samples should always be bracketed by QCs.~~ *Justification: refer to comments for line 791-797 wrt blank sample. Delete last sentence, as bracketing only makes sense for*

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		instruments on which samples are read out in a linear mode or the test system is influenced by a prolonged read time.
924-930		Comment: Harmonise wording in whole document around anchor points vs anchor calibrators *vs* anchor calibration standard Proposed change (if any):
924-930		Comment: Harmonise wording in whole document around anchor points vs anchor calibrators *vs* anchor calibration standard Proposed change (if any): EBF suggests "Anchor Points"
949-955		Comment: delete paragraph *Justification*: *During method validation it is demonstrated that samples can be diluted into the validated range. Assay range is narrow, fixed and validated for LBA For chromatography methods no change of method is needed when adjusting calibration range, however, for LBA it would mean a new method needs to be established (e.g. titration of reagents, change of reagents etc* Proposed change (if any): delete paragraph
956		Comment: Many comments came in asking for detailed clarification. An overarching theme was to consider clarifying that reanalysis is related to samples that produced valid results (e.g. >ULOQ or <LLOQ). Reanalysis of samples from failed or rejected runs that did not produce acceptable results should not be viewed as "reanalysis" in the context of this paragraph. Hence we suggest to clearly separated the examples to reflect both cases • Reanalysis of a sample which didn't give a reportable concentration, is not reanalyses per se but generates a 1st reportable result • Reanalysis of a sample for which the 1st reportable result is 'unexpected' (positive placebo, unexpected PK,…), is reanalysis. It should be performed in replicate and compared to the original result with the aim to confirm or disprove this original result. An SOP /decision tree should be in place to guide reanalysis and reporting

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any):
991		Comment: ISR = post validation = OK. During production however, ISR something else, i.e. process control, and for this you have QCs. Don't need to have multiple process controls (QC, ISR, Dil QC…) in all studies Proposed change (if any):
992-996		Comment: If the study sample shows any of the described effects which is responsible for a bias, ISR reanalysis may give the identical (biased) result. hence, ISR is related to reproducibility rather than verification of reliability of data, and accuracy and precision of the analysis. Please consider to rephrase 992-996 and focus on the fact that ISR provides information of reproducibility of the assay using real samples. Proposed change (if any):
1001-1004		Comment: rephrase Proposed change (if any): For preclinical studies, ISR should, in general, be performed for the GLP-regulated toxicokinetic studies once per species.
1005		Comment: Avoid the term "Pivotal" Proposed change (if any):
1007		Comment: Please avoid the term "pivotal" and clarify if/which Phase III studies and non-PK studies are excluded from the list. Proposed change (if any):
1009-1010		Comment: ISR analysis should be able to be conducted on the same day for methods with stability related issues.We suggest

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		adding text to discuss the ability to conduct analysis on the same day when appropriate. Proposed change (if any):
1011-1020		Comment: Please consider to provide a cap, i.e. a maximum for sample number to be analysed as part of ISR. There are strong scientific data suggesting that reanalyses of large portions of samples do not add scientific value. Literature suggests that 30 samples should be sufficient power in any study size. A consensus proposal could be: For ISR, reanalyse 10% of the study of samples, with a minimum of 20 and a maximum of 100 samples Proposed change (if any): remove % and move to min/max number of samples
1021-1023		Comment: ISR should be able to be conducted on the same day, especially if there are stability concerns. Please remove the second part of the sentence to allow ISR on the same day. → rephrase Proposed change (if any): Samples should not be pooled, as pooling may limit anomalous findings. ISR samples and QCs should be prepared in the same manner as in the original analysis. ISR should be performed within the stability window of the analyte ~~but not on the same day as the~~ but in a separate run to the original analysis.
1028-1033		Comment: This is vague and ether needs removal or clarification – suggested rephrasing Proposed change (if any): If the overall ISR results fail the acceptance criteria, an investigation should be conducted and the causes remediated. There should be an SOP that directs how any investigations are triggered and conducted. If ISR meets the acceptance criteria yet shows large or systematic differences between results for multiple samples, this may indicate analytical issues and it is advisable to investigate this further. The potential impact of an ISR investigation on study validity should be provided in the bioanalytical report.
1034		Comment: add

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): Examples of trends that are of concern may include:
1049-1050		Comment: remove – this is not a change of a method. Proposed change (if any):
1054		Comment: Rephrase Proposed change (if any): Extension of calibration range below LLOQ or above ULOQ
1060		Comment: Add Proposed change (if any): A change in sample storage conditions
1063		Comment: delete Proposed change (if any): ~~Changes in LBA critical reagents (e.g., lot-to-lot changes)~~
1065		Comment: Add Proposed change (if any): A change in sample storage conditions
1066		Comment: Rephrase Proposed change (if any): Extension of calibration range below LLOQ or above ULOQ
1068-1069		Comment: remove – this is not a change of a method.

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any):
1073		Comment: Guideline needs to better explain why cross validation is needed. Propose to add Proposed change (if any): Cross validation is conducted to evaluate the bias between methods (or laboratories) such that the results from studies using them can be appropriately interpreted. Cross validation allows the comparison of two methods (labs) and informs us how they are related.
1083		Comment: Although this is the same wording included in the EMA, it is too specific. It is just a company risk. Doing it sooner or later will not affect the quality of the data if the final cross-validation result is accepted Proposed change (if any): ~~Cross validation should be performed in advance of study samples being analysed, if possible.~~
1084-1086		Comment: rephrase Proposed change (if any): Cross validation should be assessed by measuring the same set of QCs (low, medium and high) in triplicate or study samples that span the study sample concentration range (if available n≥30) with both assays or in both laboratories
1087-1091		Comment: More discussion will be needed to understand the intention and the practical implementation of this new requirement. Not having general acceptance criteria is OK, but is new to the BA community. Hence, here needs to be systematic education all involved (industry, regulators), E.g. Who owns the decision/impact/application of correct factor? Proposed change (if any):
1095		Comment: change title Proposed change (if any): Endogenous homologue compounds

Line no.	Stakeholder no.	Comment and rationale; proposed changes
1095-1198		Comment: Industry consolidated comment = suggest rewriting this section as it is too detailed and prescriptive. Proposed change (if any):
1095-1198		Comment: in addition to above comment, EBF suggests that the re-written section at least contains the "background addition approach" Proposed change (if any):
1200-1215		Comment: rephrase Proposed change (if any): …is suspected during study sample analysis. ~~Parallelism investigation or the justification for its absence should be included in the Bioanalytical Report.~~ Where assessed, parallelism investigation should be reported. As parallelism assessments ….. should be defined a priori.
1217-1225		Comment: add Proposed change (if any): For methods that employ sample extraction, the recovery (extraction efficiency) should be evaluated during method development.
1238-1241		Comment: rephrase Proposed change (if any): If an applicant uses a kit, repurposes a kit (instead of developing a new assay) or utilises "research use only" kits to measure chemical or biological drug concentrations during the development of a novel drug, the applicant should ~~assess the kit~~ perform a validation to ensure …
1242		Comment: rephrase

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): Validation specific considerations for kit assays include, but are not limited to, the following:
1243-1247		Comment: Add *Justification: As described in this guideline, the same principles of validation apply to the use of kits and validation should be performed under actual conditions of use in the facility conducting the sample analysis.* Proposed change (if any): Kits components should be considered as a source of critical reagents (refer to Section 4.1.2)
1248-1250		Comment: remove Proposed change (if any):
1254-1256		Comment: Suggest to allow same flexibility in use of surrogate/alternative matrix for preparation of calibration standards and QCs for standard PK assays (see section 4.2.3) as it is allowed for kits described in section 7.5 as long as the use is verified and justified. Proposed change (if any):
1259-1261		Comment: remove Proposed change (if any):
1265-1273		Comment: Replace the sentence describing "cross validation" to reflect that the two techniques/technologies should be compared to understand and establish the correlation between the two measurements while being mindful that the two measurements can give different values. Proposed change (if any):

Line no.	Stakeholder no.	Comment and rationale; proposed changes
1276-1300		Comment: This section is too specific and relates only to dried blood spot sampling. The suggested revisions - below- enables the inclusion of all/other dried matrix sampling techniques/technologies Proposed change (if any):
1277-1281		Comment: delete Proposed change (if any): Dried matrix methods (DMM) is a sampling methodology that offers benefits such as collection of reduced blood sample volumes ~~as a microsampling technique~~. In addition to the typical methodological validation for LC-MS or LBA, use of DMM necessitates further assessment of this sampling approach before using DMM in studies that support a regulatory application, such as:
1282		Comment: delete Proposed change (if any): Haematocrit ~~(especially for spotting of whole blood into cards)~~
12831284		Comment: delete Proposed change (if any): Sample homogeneity ~~(especially for sub-punch of the sample on the card/device)~~
1285		Comment: rephrase Proposed change (if any): ~~DMM sample collection for ISR~~ Consideration for being able to conduct ISR
1286-1287		Comment: remove Proposed change (if any):

Line no.	Stakeholder no.	Comment and rationale; proposed changes
1288-1289		Comment: remove Proposed change (if any):
1290-1293		Comment: rephrase Proposed change (if any): When DMM is used for clinical or nonclinical studies in addition to typical liquid approaches (e.g., liquid plasma samples) ~~in the same studies, these two methods should be cross validated as described (Refer to Section 6.2). For nonclinical~~ the comparability of the two methods should be determined using a priori defined correlation approach. In addition, for nonclinical TK studies, refer ~~to Section 4.1 of~~ ICH S3A Q&A. Feedback from the appropriate regulatory authorities is encouraged in early drug development.
1345		Comment: add Proposed change (if any): Accuracy (%) as defined by relative error = Measured Value/Nominal Value x 100
1393-1396		Comment: new definition Proposed change (if any): The calibration range of an analytical procedure is the interval between the LLOQ and ULOQ of the calibration curve (excluding any anchor point samples) for which it has been demonstrated that the analytical procedure meets the requirements for precision, accuracy and response function.
1408-1410		Comment: new definition Proposed change (if any): Reagents, including binding reagents (e.g. binding proteins, aptamers, antibodies or conjugated antibodies), that have direct impact on the results of the assay and therefore their quality must be assured.
1419-1422		Comment: new definition

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change (if any): A parameter demonstrating that the method can appropriately analyse samples at a concentration exceeding the ULOQ of the calibration curve when diluted within the calibration range in LBAs.
New to glossary		Comment: add individual definitions to unambiguously define "sample" " aliquot" and "Replicate Analysis or Measurement" Proposed change (if any): suggestions given • Sample = a quantity (of something) from which the general quality (of the whole) may be inferred • Aliquot = any representative portion of the sample • Replicate Analysis or Measurement = The repeated analysis or measurement of the variable of interest performed as identically as possible.
New to glossary		Comment: add individual definitions to unambiguously define "fresh" Proposed change (if any): suggestion given • Prepared on the day of analysis or analysed within stability and the using intermediates which are within known stability (or to be proven stability).'
New to glossary		Comment: add individual definitions to unambiguously define "initial integration" and "reintegration", which are quite distinct processes and need clear definition and control. Proposed change (if any): suggestions given • Initial integration is defined as the process by which the area (or height) of a chromatographic peak is adequately defined by trained personnel using the most appropriate parameters prior to regression. (consider GBC S1-3 recommendation paper) • Reintegration: any changes, either automatic or manual, applied to individual chromatograms after having established the integration parameters for the run. Re-integration is applied prior to regression and/or calculation of concentrations to

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		ensure non-bias of the process.
New to glossary		Comment: add individual definition to unambiguously define "geometric mean" Proposed change (if any): suggestion given • Geometric mean is defined as the square root of product of LLOQ and ULOQ
New to glossary		Comment: add individual definition to unambiguously define "primary matrix" Typically, a study has one primary matrix. Additional matrices should be considered as non-primary matrix. Only in rare cases a study can have multiple primary matrices. It is recommended the primary matrix(ces) is/are clearly defined in the protocol. Proposed change (if any):
New to glossary		Comment: add individual definition to define "Reference Standard" Proposed change (if any): suggestion given • A well-characterised substance used to prepare calibration and quality control samples. A reference standard should be accompanied by a certificate of analysis or equivalent documentation to prove identity, purity and stability (expiration or retest date)
New to glossary		Comment: add individual definition to define "Dilution ratio (or factor)" Proposed change (if any): suggestion given

<table>
<tr><th>Line no.</th><th>Stakeholder no.</th><th>Comment and rationale; proposed changes</th></tr>
<tr><td></td><td></td><td>• The ratio of sample to diluent used to dilute the sample. Also referred to as a dilution factor.</td></tr>
<tr><td>1294</td><td></td><td>Comment: As per our general comment on page 2, we propose to add an additional column, specifically for BA/BE studies. The requirement for reporting for the other studies could be simplified and focus on ‘documentation at the analytical site’ rather than intensive reporting in Bioanalytical reports

Proposed change (if any):</td></tr>
<tr><td>Documentation table 1</td><td></td><td>Comment: Proposed changes to table 1, section “Documentation at the Analytical Site” applicable for BA/BE studies

<table>
<tr><th>Items</th><th>Documentation at the Analytical Site</th><th>Proposed changes or comments</th></tr>
<tr><td>Blank Matrix</td><td>• Records of matrix descriptions, lot numbers, receipt dates, storage conditions, and source/supplier</td><td>Change to – “Description”</td></tr>
<tr><td>Sample Tracking</td><td>• Records that indicate how samples were transported and received. Sample inventory and reasons for missing samples</td><td>Add: Sample inventory and, where available, reasons for missing samples</td></tr>
<tr><td>Audits and Inspections</td><td>• Audit and inspection report</td><td>QA audit reports are not shared</td></tr>
</table></td></tr>
<tr><td>Documentation table 1</td><td></td><td>Comment: Proposed changes to table 1, section “Validation Report” applicable for BA/BE studies

<table>
<tr><th>Items</th><th>Validation Report*</th><th>Proposed changes or comments</th></tr>
<tr><td>Calibration Standards and QCs</td><td>• Batch number, preparation dates and stability period</td><td>Change to – “A list of analytical procedure(s)”</td></tr>
<tr><td>Analysis</td><td>• Instrument ID for each run in comparative BA/BE studies</td><td>Delete</td></tr>
</table></td></tr>
</table>

Line no.	Stakeholder no.	Comment and rationale; proposed changes

Analysis	• Table of calibration standard concentration and response functions results (calibration curve parameters) of all accepted runs with accuracy and precision.	Change to – "Table of calibration standard concentration and response functions results (~~all~~ applicable calibration curve parameters) of all runs, with accuracy and precision of accepted runs"
Analysis	• Data on selectivity (matrix effect), specificity (interference), dilution linearity and sensitivity (LLOQ), carry-over, recovery. Bench-top, freeze-thaw, long-term, extract, and stock solution stability	change to: Bench-top, freeze-thaw, long-term, extract, and stored working solution stability
Chromatograms and Reintegration	• For comparative BA/BE studies, 100% chromatograms of original and reintegration from accepted and fail runs.	Does not belong in the validation report
Chromatograms and Reintegration	• Chromatograms may be submitted as a supplement	Does not belong in the validation report
Chromatograms and Reintegration	• For comparative BA/BE studies,100% of run summary sheets of accepted and failed runs, including calibration curve, regression, weighting function, analyte and IS responses and retention times and dilution factor if applicable.	Provide example of run summary sheet

Documentation table 1

Comment: Proposed changes to table 1, section "Bioanalytical Report" applicable for BA/BE studies

Items	Bioanalytical Report*	Proposed changes or comments
Blank Matrix	• Description, lot number, receipt dates[††]	remove receipt dates; kept at the analytical site
Sample Tracking	• Dates of receipt of shipments number of samples, and for comparative BA/BE studies the subject ID	Change to – "For comparative BA/BE studies, dates of receipt of shipments, number of samples and subject ID"
Sample Tracking	• Analytical site storage condition and location	Change to – "Analytical site storage condition"
Sample Tracking	• List of any deviations from planned storage conditions, and potential impact	Change to – "List of any deviations from planned storage conditions that impacted on study results"
Analysis	• Instrument ID for each run in comparative BA/BE	Delete

Line no.	Stakeholder no.	Comment and rationale; proposed changes		
			studies	
		Analysis	• Table of QCs results of all accepted runs with accuracy and precision results of the QCs and between-run accuracy and precision results from accepted runs.	**Change to –** "Table of QCs results of all runs with accuracy and precision results of the QCs and between-run accuracy and precision results from accepted runs."
		Chromatograms and Reintegration	• For comparative BA/BE studies, 100% of run summary sheets of accepted and failed runs, including calibration curve, regression, weighting function, analyte and IS responses and retention times, and dilution factor if applicable.	Provide example of run summary sheet

Please add more rows if needed.