ANIMAL HEALTH INSTITUTE
Representing manufacturers of animal health products

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Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, rm. 1061
Rockville, MD 20852

Re: Docket No. FDA–2013–D–1020; Draft Guidance for Industry on Bioanalytical Method Validation

The ANIMAL HEALTH INSTITUTE ("AHI") submits these comments to FDA–2013–D–1020; Draft Guidance for Industry on Bioanalytical Method Validation**.** AHI is the national trade association representing manufacturers of animal health products -- the pharmaceuticals, biological products and feed additives used in modern food production, and the medicines that keep livestock and pets healthy. AHI member companies represent the majority of animal pharmaceuticals and animal insecticides, as well as serving a significant segment of the world market.

We are not sure of the value of having a joint CDER and CVM guideline since only a small portion of this guidance applies to CVM. We see the potential for disconnect in the long term since the scope of this guidance is limited to PK BE studies on the veterinary side. ICH is referenced frequently, but we have been working under the points to consider included in the Crystal City 3 White Paper guidelines up to this point in the absence of a VICH Bioanalytical standard. A lot of points included in the Crystal City 3 White Paper are not included in the current proposed guidance. We recommend that all parts that are missing be reincorporated and that this guidance be published again for comment prior to finalizing.

Please see the attached table for specific comments and recommendations on the draft guidance. A majority of our comments could be resolved by simply adopting more parts of the prior guidelines currently being followed (Crystal City 3 White Paper).

Thank you in advance for your consideration of these comments. Should you have any questions, please do not hesitate to contact AHI at (202) 637-2440.

Sincerely,

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Commenter	**Page No.**	**Line(s)**	**Current Text**	**Comments/Recommendations/Questions**
AHI	General Comment			CVM is partnering with CDER (rather than having different sets of guidances). Of particular note to us - the proposed guidance requires incurred sample re-analysis as part of Bioanalyt validation and performance schema. LBAs are specifically mentioned in the same guidance as chromatographic methods.
AHI	General Comment			We ask that CVM consider the Crystal City 3 White Paper, in more detail, then appears to have been done with current version of this draft guideline. In addition, as CVM is aware, the VICH GL 52 (Bioequivalence) has a listing of required parameters, but no information regarding limits, values, etc. We suggest that this guideline be considered with the idea that it could be harmonized within VICH so that methods developed and validated to support Bioequivalence studies would be accepted in VICH countries.
AHI	General Comment			Guidance to the different validation parameters is, at some points, scattered throughout the document (e.g. Use of QCs is mentioned in IV.B.2 under Accuracy, Precision and Recovery but also in IV.B.3.d under Calibration Curve. The definition of Sensitivity at IV.B.4 seems to refer to a QC but is not clear). Also, definitions as presented in IV.B.4 Sensitivity and IV.B.5 Reproducibility seem to refer to earlier described sections, but this is not clear. We suggest that the validation parameters be clustered and clear for each section; if a parameter needs to be reiterated in a subsequent section then cross-reference as to clarify.
AHI	Page 1	Lines 23-26	For studies related to the veterinary drug approval process (Investigational New Animal Drug Applications (INADs), New Animal Drug Applications (NADAs), and Abbreviated New Animal Drug Applications (ANADAs)), this guidance may apply to blood and urine BA, BE, and PK studies.	The scope needs to be clearly defined with respect to the veterinary drug approval process. The use of the term “may” is unclear.

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AHI	Page 1	Lines 28-33	The information in this guidance generally applies to bioanalytical procedures, such as gas chromatography (GC); high-pressure liquid chromatography (LC); combined GC and LC mass spectrometric (MS) procedures, such as LC-MS, LC-MS-MS, GC-MS, and GC-MS-MS; and ligand binding assays (LBAs), and immunological and microbiological procedures that are performed for the quantitative determination of drugs and/or metabolites, and therapeutic proteins in biological matrices, such as blood, serum, plasma, urine, tissue, and skin.	The relevance of this guidance to tissue assays should be clarified so that residues in edible tissues are explicitly be excluded.
AHI	Page 2	Lines 77-78	For exploratory methods used for the sponsor's internal decision making, less validation may be sufficient.	We recommend omitting this line. It is overly prescriptive. It is obvious that if the study is being performed for internal decision making only, this guidance doesn't apply.
AHI	Page 2	Line 105	Bioanalytical method transfers between laboratories or analysts	We do not agree that an analyst change requires a partial validation method. Method ruggedness can be evaluated by having one validation day performed by another analyst. (This is true but is not in the guidance). It would be an overkill to have re-validation whenever an operator changes within the same laboratory.
AHI	Page 3	Line 107	Change in anticoagulant in harvesting biological fluid (e.g., heparin to EDTA)	It is not clear if additional experiments are needed if the anticoagulant counter-ion is changed, for example, sodium heparin to lithium heparin or K3 to K2-EDTA.
AHI	Page 3	Line 107 Line 108 Line 110	Change in anticoagulant in harvesting biological fluid (e.g., heparin to EDTA) Change in matrix within species (e.g., human plasma to human urine) Change in species within matrix (e.g., rat plasma to mouse plasma)	Some qualifying language may be necessary to indicate that partial revalidation can be used "when appropriate" to allow for this expectation.
AHI	Page 4	Lines 133-135	The analytical laboratory conducting nonclinical pharmacology/toxicology studies for regulatory submissions should adhere to FDA's Good Laboratory Practices (GLPs) requirements (21 CFR Part 58).	We recommend specifying that a validation should be run as a GLP study.
AHI	Page 4	Lines 162-163	Three types of reference standards are usually used: (1) certified reference standards (e.g., USP compendial standards)…	USP reference standards do not list expiry dates; there will be no reference to when the standard expires for re-establishment of purity and would result in GLP findings during an audit.

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AHI	Page 4	Lines 165-168	The source and lot number, expiration date, certificates of analyses when available, and/or internally or externally generated evidence of identity and purity should be furnished for each reference and internal standard (IS) used.	The requirements for COAs/identity/purity assessments for internal standards appear to be contrary to the Crystal City 3 White Paper. Should reference standards be archived in the same manner as other GLP samples/documentation?
AHI	Page 4	Lines 168-169	If the reference or internal standard expires, stock solutions made with this lot of standard should not be used unless purity is re-established.	The Crystal City 3 White Paper specifically stated that expiration dates for solids are not extrapolateable to solutions. It would not be practical if the reference standard expires in the middle of a study to discard all the solutions and wait for a new incoming batch. Moreover, stability in solid state is different (e.g. hygroscopic substances). We suggest adding the word “new” before “stock solutions” to read: *If the reference or internal standard expires,* ***new*** *stock solutions made with this lot of standard should not be used unless purity is re-established.*
AHI	Page 5	Lines 174-177	Each step in the method should be investigated to determine the extent to which environmental, matrix, or procedural variables could affect the estimation of analyte in the matrix from the time of collection of the samples to the time of analysis.	It seems excessive to investigate each step. The laboratory should evaluate the method and determine which parameters are critical. Critical parameters should be investigated. While the Bioanalytical (BA) Group is responsible for developing sample collection conditions, monitoring of sample collection at clinical sites is outside the scope of most BA groups and the in-life protocol would describe these environmental conditions.
AHI	Page 5	Lines 196-199	Analyses of blank samples of the appropriate biological matrix (plasma, urine, or other matrix) should be obtained from at least six sources. Each blank sample should be tested for interference, and selectivity should be ensured at the lower limit of quantification (LLOQ).	We recommend the criteria for selectivity from the Crystal City 3 White Paper be added back into the guidance.

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AHI	Page 5	Lines 211-212	A minimum of three concentrations in the range of expected study sample concentrations is recommended.	If the study sample concentration is over the calibration range, there is no way to use three concentrations above the range to assess accuracy and precision. We strongly suggest revising the guidance to read: *"A minimum of three concentrations **in the calibration range** is recommended."* For the validation, QC prepared at the LLOQ should be included as required by the EMEA guidelines.
AHI	Page 6	Line 221-223	The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV.	This appears to be a typo. The sentence should read: *The precision **(coefficient of variation, CV)** determined at each concentration level should not exceed 15% of the ~~coefficient of variation (CV)~~ **mean** except for the LLOQ, where it should not exceed 20% of the ~~CV~~ **mean**.*
AHI	Page 6	Lines 224-227	*Within-run precision* (intra-batch precision or within-run repeatability) is an assessment of precision during a single analytical run. *Between-run precision* (inter-batch precision or between-run repeatability) is an assessment of precision over time and may involve different analysts, equipment, reagents, and laboratories.	We suggest a minimum of three between-run precision measurements. More clarity is required here on run numbers and acceptance criteria for batches.
AHI	Page 6	Line 229	Sample concentrations above the upper limit of the standard curve should be diluted.	The guidance should provide criteria for such dilution since dilution factor can also be lower / higher than validated, but needs to be checked with QCs at the same dilution factor. We recommend adding the following sentences: *In cases where samples need to be diluted to fall within the calibration range, diluted QC samples should be prepared using the same matrix and assessed during method validation. The diluted QC is validated via a single accuracy and precision run in which the desired dilution is applied to 6 separate aliquots on 3 separate occasions. The accuracy and precision criteria are the same as for other QCs. Additional dilutions can be qualified following validation in just one analytical run in which 6 replicates are evaluated as described above.*

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AHI	Page 6	Lines 233-240	The *recovery* of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the analyte in solvent. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible. Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards that represent 100% recovery.	The Agency should clarify, given that *recovery*, as used here, seems to suggest a combination of extraction recovery and matrix effect. Determining recovery versus analyte in solvent confounds matrix effects with recovery. Recovery should be calculated versus analyte spiked into extracts of blank matrix samples. Matrix effects on ionization are excluded from discussion. We recommend adding a section on matrix effect assessment from the Crystal City 3 White Paper. Is there an expectation for minimum replicates per standard? The last sentence should be changed to read: *Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with ~~unextracted~~* ***neat*** *standards that represent 100% recovery.*
AHI	Page 6	Lines 251-256	Concentrations of standards should be chosen on the basis of the concentration range expected in a particular study. A calibration curve should consist of a blank sample (matrix sample processed without analyte or internal standard), a zero sample (matrix sample processed without analyte but with internal standard), and at least six non-zero samples (matrix samples processed with analyte and internal standard) covering the expected range, including LLOQ.	Blank sample and zero sample should not be counted in the calibration curve; instead it should be stated that these samples should be included in an analytical run.
AHI	Page 6	Lines 258-260	Method validation experiments should include a minimum of six runs conducted over several days, with at least four concentrations (including LLOQ, low, medium, and high) analyzed in duplicate in each run.	What is the difference between this and the between-run precision? The Agency should provide justification/clarification for the need of six runs for method validation, as precision and accuracy during validation is generally assessed in three runs. For clarification, need to separate the core run to assess the accuracy and precision (intra-day) and the validation runs to assess other parameters such as stability. Please clarify if calibrants need to be prepared in duplicate or if they are reanalyzed in duplicate and the number of runs required.
AHI	Page 7	Lines 280-282	Analyte peak (response) should be reproducible and the back-calculated concentration should have precision that does not exceed 15% of the CV and accuracy within 15% of the nominal concentration.	The Agency should clarify if this means the QC at ULOQ has to be prepared. If it is only from the curve, there will be no way to assess intra-day %CV.

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AHI	Page 7	Lines 291-295	The acceptance criterion for the standard curve is that at least 75% of non-zero standards should meet the above criteria, including the LLOQ. Excluding an individual standard should not change the model used. Exclusion of calibrators for reasons other than failing to meet acceptance criteria and assignable causes is discouraged.	Standard curve should include at least 75% non-zero standards or a minimum of 6, whichever is greater. We suggest adding that standard curve should consist of a minimum of 6 non-zero points. The ability to drop the LLOQ standard during daily runs and adjust the daily LLOQ of the assay, as long as there is at least one standard below the Low QC, is not clear. The guidance should clarify whether it is permissible to drop the LLOQ standard during daily runs.
AHI	Page 7	Lines 297-302	d. Quality Control Samples (QCs) At least three concentrations of QCs in duplicate should be incorporated into each run as follows: one within three times the LLOQ (low QC), one in the midrange (middle QC), and one approaching the high end (high QC) of the range of the expected study concentrations.	This section is confusing as it is not clear if it refers of routine use of the validated analytical method or to validation experiments.
AHI	Page 8	Lines 329-331	*Reproducibility* of the method is assessed by replicate measurements using the assay, including quality controls and possibly incurred samples. Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument interruptions.	The Agency should further provide clarity and stipulate the number of replicates.
AHI	Page 8	Lines 336-338	Pre-study stability evaluations should cover the expected sample handling and storage conditions during the conduct of the study, including conditions at the clinical site, during shipment, and at all other secondary sites.	It is unclear if stability demonstration on “dry ice” is needed to demonstrate stability during shipment. We believe stability should be assessed over the temperature range that samples are expected to be exposed to prior to analysis. Would this include short-term stability of analyte in blood prior to processing for plasma?
AHI	Page 8	Lines 341-343	The stability of an analyte in a particular matrix and container system is relevant only to that matrix and container system and should not be extrapolated to other matrices and container systems.	The term “container system” is ambiguous. Does this refer to container material or a specific brand of container? The guidance should clarify the meaning of the term “container system”. More clarity and justification is needed to determine if and when a partial validation needs to be done for supplier changes. It seems unnecessary to conduct a partial validation just because the supplier of a 96-well plate was changed.

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AHI	Page 9	Lines 353-355	The procedure should also include an evaluation of analyte stability in stock solution. All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix.	The current statement implies that the stability of stock solutions should be tested as a matrix spiked sample. Such spiking is not required for testing pure analyte. Stock and working solutions can be tested by injection of analyte in mobile phase.
AHI	Page 9	Lines 353-359	The procedure should also include an evaluation of analyte stability in stock solution. All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix. Stock solutions of the analyte for stability evaluation should be prepared in an appropriate solvent at known concentrations. Stability samples should be compared to freshly made calibrators and/or freshly made QCs. At least three replicates at each of the low and high concentrations should be assessed. Stability sample results should be within 15% of nominal concentrations.	Stability investigations of analytes in matrix should be conducted with incurred samples if available as these are the real samples. Spiked samples could be used as an alternative. However, care should be taken to not to add too much organic solvent as the metabolizing enzymes could be destroyed. What about the stability of the working solution? Does this need to be evaluated? This paragraph needs to make a distinction between stock solution stability and other stability criteria. We believe stability determinations should use freshly made stock solution of analyte. Is the stability based on the mean of the samples, or on the individual values of the three samples? These lines are quite confusing. It is not clear whether it refers to stability determination (i.e., freeze & thaw, bench top, etc) or only to stock solution stability. The paragraph “d” for stock solution stability should include guidance and acceptance criteria for such experiments. A clear distinction should be established with stability determination in matrix.
AHI	Page 9	Line 359	Stability sample results should be within 15% of nominal concentrations.	We would like to see the stability result compared to the initial result added. If the initial result is +15% a total of 30% degradation would be permissible under this guidance. Likewise, if the initial result is only -15% then even a degradation of 1% would be impermissible.
AHI	Page 9	Lines 374-375	The storage time in a long-term stability evaluation should equal or exceed the time between the date of first sample collection and the date of last sample analysis.	Shouldn’t this be the longest storage time of any study sample? What about long term studies where enrollment of patients onto the study may occur over the course of a year or more but each patients’ samples are collected and analyzed within a shorter period of time?
AHI	Page 9	Line 379	The stability of stock solutions of drug and internal standard should be evaluated.	Internal standards should only be assessed for suitability. Stability of internal standard stocks should not be recommended.

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AHI	Page 9	Lines 379-383	When the stock solution exists in a different state (solution vs. solid) or in a different buffer composition (generally the case for macromolecules) from the certified reference standard, the stability data on this stock solution should be generated to justify the duration of stock solution storage stability.	Could more detail regarding stable isotope-labeled internal standards be included? In this case chemical and isotope stability are an issue.
AHI	Pages 9-10	Lines 395-398	System suitability: If system suitability is assessed, a specific SOP should be used. Apparatus conditioning and instrument performance should be determined using spiked samples independent of the study calibrators, QCs, or study samples. Data should be maintained with the study records.	The guideline should not insist on matrix based solvent to test system suitability. This parameter can be evaluated with solvent based samples.
AHI	Page 10	Lines 402-408	If the bioanalytical method necessitates separation of the overall analytical run into distinct processing batches (e.g., capacity limit of 96-well plates or solid phase extraction manifold, extraction by multiple analysts), each distinct processing batch should process at least duplicates QCs at all QC levels (e.g., low, middle, high) along with the study samples. In such cases, acceptance criteria should be established for the analytical run as a whole as well as the distinct processing batches.	The need for a full set (2 of each level) of QCs on each plate of a multi plate run is unclear. It is generally understood that partial run acceptance is not appropriate, thus the need for acceptance criteria for each processing batch should be eliminated. The part of sentence "*as well as the distinct processing batches*" should be removed.
AHI	Page 10	Line 416	Concentrations below the LLOQ should be reported as zeros.	Shouldn't this be defined in the protocol? It seems like this may be a decision that will be specific to the application. We report <LLOQ as ½ LLOQ. We've also seen them reported as <LLOQ. It is up to the statistician to decide how to treated values <LLOQ not the BA lab. This sentence should be deleted from the guidance.
AHI	Page 10	Line 417	Any required sample dilutions should use like matrix (e.g. human to human).	We recommend the following change: *Any required sample dilutions should use like matrix (e.g. human to human)* ***unless validated otherwise****.*
AHI	Page 10	Lines 423-425	Changes in the response function relationship between pre-study validation and routine run validation indicate potential problems.	"Routine run validation" should be defined in the guidance for clarity.
AHI	Page 10	Lines 425-426	Internal standard response should be monitored for drift. An SOP should be developed *a priori* to address issues related to variability of the IS response.	This point should be clarified by the Agency. Drift of internal standard should not be monitored, QCs samples are there to determine if the method performance fulfill the acceptance criteria But it is true that IS response and changes in Rt is now used widely as a check of assay performance even though the QC have passed the run..

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AHI	Page 10	Lines 432-436	If the study sample concentrations are clustered in a narrow range of the standard curve, additional QCs should be added to cover the sample range. Accuracy and precision of the additional QCs should be validated before continuing with the analysis. If the partial validation is acceptable, samples that have already been analyzed do not require re-analysis.	We don't understand the scientific rationale behind validating QCs in the middle of the range. If the new QC concentrations are bounded by previously validate QC concentrations, those new concentrations should not need to be validated because they are in the validated range. It is difficult to know a priori what the concentration range of the samples is going to be. Are samples to be re-run with new QC concentrations if their concentrations are clustered? We recommend striking this whole paragraph.
AHI	Page 10	Line 437	All study samples from a subject should be analyzed in a single run.	This expectation would sometimes be impossible to achieve as it would require stability assessment for longer period if the study is a long-term study. An exception should be specified for long multiple phased studies.
AHI	Page 11	Lines 438-439	Carryover should be assessed and monitored during analysis. If carryover occurs, it should be mitigated or reduced.	The guidance should provide acceptance criteria for this carryover recommendation. Does the monitoring of carryover refer to the validation process or to the analysis of study samples?
AHI	Page 11	Lines 445-446	Reassays should be done in triplicate if sample volume allows.	We question the need for recommending triplicate reassays. In many cases, duplicate reassays should be sufficient. It would be better to require a discussion of the rationale for the number of aliquots required.
AHI	Page 11	Lines 448-449	Samples involving multiple analytes should not be rejected based on the data from one analyte failing the acceptance criteria.	This statement could be reworded for clarity. i.e. "*In cases where samples are analyzed for more than one analyte, the failure of a single analyte to meet acceptance criteria should not necessarily result in the rejection of the data from other analytes that fall within the acceptance criteria.*" The assumption is that this statement applies only to individual samples and not to batches of samples. If not, please clarify.
AHI	Page 11	Lines 452-455	If a unique or disproportionately high concentration of a metabolite is discovered in human studies, a fully validated assay may need to be developed for the metabolite, depending upon its activity (refer to the FDA guidance for industry *Safety Testing of* Drug *Metabolites*).	This is outside of the scope – addressed elsewhere. Current technologies (i.e. mass spec) may or may not appropriately capture metabolites in a sample. But if they do, and are then to be quantified, they will require a validated method.
AHI	Page 11	Lines 462-463	Audit trails should be maintained. Original and reintegration data should be reported.	Expecting that re-integration data be reported is confusing. We suggest that original and reintegration data should be retained as part of the raw data.

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AHI	Page 16	Lines 665-666	Stock solution stability also should be assessed. Stability sample results should be within 15% of nominal concentrations.	For ligand binding assays on a large molecule, 20% would be more typical than 15%.
AHI	Page 17	Lines 705-706	Accuracy and precision as outlined in Section IV.B.2 should be provided for both the inter-run and intra-run experiments and tabulated for all runs (passed and failed).	This statement is more relevant for validation experiments. For routine analysis the acceptance criteria are defined and should be applied. It is not necessary to calculate inter and intra-run accuracy and precision in such case.
AHI	Page 17	Line 742	The data from rejected runs should be documented, but need not be reported;	This is in contradiction with lines 705-706 which state: *Accuracy and precision as outlined in Section IV.B.2 should be provided for both the inter-run and intra-run experiments and tabulated for all runs (passed and failed).* Please clarify.
AHI	Page 18	Line 750	V. INCURRED SAMPLE REANALYSIS	The minor deviations from the current EMA guidance are not very helpful in global application of the guidances (e.g. select 7% instead of 5%). We strongly suggest synchronizing these ISR guidances. If this topic is not synchronized it will continue to be point of discussion.
AHI	Page 18	Lines 757-758	ISR samples should be compared to freshly prepared calibrators.	The use of the term “freshly prepared” is ambiguous. It is more appropriate to use calibrators that are within established stability period. This will always hold true since the ingredients of the calibrators will have been stored. We suggest you revise the sentence to read: *ISR samples should be compared to freshly ~~prepared~~ **extracted** calibrators.*
AHI	Page 18	Lines 758-759	ISR is expected for all in vivo human BE studies and all pivotal PK or pharmacodynamic (PD) studies.	As worded, the statement focuses on human drug development does not take into account the range of studies performed for veterinary development (for instance, veterinary BE studies for generic animal drugs or PK/PD for dose justification in the intended species). Further clarification on when ISR would be appropriate for studies intended for veterinary development should be included in this statement.
AHI	Page 18	Lines 765-766	For applications with a greater number of pivotal PK or PD studies, ISR should be monitored in a larger number and variety of studies.	This is very vague. In case a method appears to be appropriately performing in the defined type of clinical study, it does not seem to be helpful to repeat that in more studies with the same scope.
AHI	Page 18	Line 771	The total number of ISR samples should be 7% of the study sample size.	We strongly suggest the guidance follow EMA GUI 10% or 5% if less than 1000.

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AHI	Page 18	Line 775-776	Two-thirds (67%) of the repeated sample results should be within 20% for small molecules and 30% for large molecules.	This statement is restrictive. While large molecules are almost exclusively analysed by LBA, not all small molecules are analysed by chromatography; there are still some small molecules that are analysed by LBA. We suggest you reword the sentence to read: *Two-thirds (67%) of the repeated sample results should be within 20% for small molecules and 30% for ~~large molecules~~* ***LBA analytes****.*
AHI	Page 21	Line 911	Method development and validation data and reports.	We don't write method development reports.
AHI	Page 22	Line 925-926	Information related to each bioanalytical run should be maintained at the laboratory and should include the analysts performing the run, start and stop times (duration), raw data, integration codes, and/or other reporting codes.	We suggest you reword the sentence to include: *Information related to each bioanalytical run should be maintained at the laboratory* ***or archive location****, and should include the analysts performing the run, start and stop times (duration) raw data, integration codes, and/or other reporting codes.*
AHI	Page 22	Lines 930-932	Data generated from system suitability checks should be maintained in a specific file on-site and should be available for inspection.	We recommend retaining study specific system suitability test file and archiving it with study raw data.
AHI	Page 23	Lines 961-962	Evidence of purity and identity of drug, metabolites, and IS used at the time of the validation experiments.	Only suitability of internal standard should be recommended by the guidance. We suggest dropping the recommendation for evidence of purity and identity of IS.
AHI	Page 23	Lines 969-970	QCs results that fail to meet the acceptance criteria should not be excluded from calculations of accuracy and precision unless there is an assignable cause.	In case the calibration curve does not meet the acceptance criteria there is no use in reporting the QC data either. The guidance should acknowledge (with appropriate rationale) that "failed calibration curve" is an acceptable assignable cause.
AHI	Page 23	Lines 989-990	All measurements with the individual calculated concentrations should be presented in the validation report.	All results with the individual calculated concentrations should be presented in the validation report.
AHI	Page 23	Lines 996-997	Evidence of purity at the time of use and identity of drug standards, metabolite standards, and internal standards used during routine analyses, and expiration or retest dates.	Only suitability of internal standards should be recommended by the guidance. We suggest dropping the recommendation for purity and identity of internal standard.

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AHI	Page 24	Lines 999-1001	Sample identification, collection dates, storage prior to shipment, information on shipment batch, and storage prior to analysis. Information should include dates, times, and sample condition.	Bioanalytical laboratories generally do not have access to information on individual samples prior to their arrival in the bioanalytical laboratory. In general, the information is captured in other study records; therefore, it is inappropriate to expect such information to be with Bioanalytical records when Bioanalytical laboratory is not responsible for documenting and maintaining such records. The guidance should delete “storage prior to shipment” and include a statement regarding expectations for the longest period elapsed between sample collection and analysis.
AHI	Page 24	Lines 1002-1003	Any deviations from the validated method, significant equipment and material changes, SOPs, protocols, and justifications for deviations.	In the case where the analysis is being performed for a GLP report, not only should these be documented in the report, but there is a need for communication to the Study Director regarding the deviation and impact contemporaneous with the study is needed. Should this be mentioned?
AHI	Page 24	Line 1011	Reasons for missing samples.	Bioanalytical laboratories generally do not have information regarding missing samples. We strongly suggest deleting the “Reasons for missing samples” bullet.
AHI	Page 24	Lines 1012-1014	Repeat analyses should be documented with the reason(s) for the repeat analysis, the initial and repeat analysis results, the reported result, assay run identification, and the manager authorizing reanalysis.	This should be the responsibility of the person in charge of the bioanalysis (often referred to as Principal investigator). “Manager” can be interpreted as the “people manager,” which seems inappropriate to authorize a sample re-analysis. Please define "manager" is this test facility management, laboratory management or Study Director? Or is this SOP-defined. We recommend omitting “manager authorizing” from line 1014. It is overly prescriptive.
AHI	Page 24	Lines 1014-1015	Repeat analysis of a clinical or nonclinical sample should be performed only under a predefined SOP.	What about reanalysis guidelines that may be specific to a particular study and defined in the protocol? Should this be written as a study-specific SOP, or is specification as the protocol superseding the SOP sufficient?

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Commenter	**Page No.**	**Line(s)**	**Current Text**	**Comments/Recommendations/Questions**
AHI	Page 24	Lines 1016-1020	Data from reintegrated chromatograms should be documented with the reason for reintegration, initial and repeat integration results, the method used for reintegration, the reported result, assay run identification, and the manager authorizing reintegration. Reintegration of a clinical or nonclinical sample should be performed only under a predefined SOP.	The guidance should not recommend including re-integration information in the bioanalytical report. In addition, re-integration should be specific to those chromatograms using integration parameters different from others in the batch. We believe data from re-integrated chromatograms should be maintained in the study file. We recommend omitting “manager authorizing” from line 1018. It is overly prescriptive.