17 November 2011
EMA/CHMP/643484/2011
Committee of Medicines for Human Use (CHMP)

Overview of comments received on 'Appendix IV of the Guideline on the Investigation on Bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1): Presentation of Biopharmaceutical and Bioanalytical Data in Module 2.7.1 (EMA/CHMP/600958/2010)'

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	European Generic medicines Association (EGA)
2	European Federation of Pharmaceutical Industries and Associations (EFPIA)
3	The Association of the European Self-Medication Industry (AESGP)
4	BEBAC – Helmut Schütz
5	European Bioanalysis Forum
6	Hexal AG
7	MSD, a subsidiary of Merck & Co. Inc.
8	Mundipharma Research
9	Perrigo (UK)
10	Pharmaceutical Research Institute, (Warsaw, Poland)
11	SciencePharma sp. z o.o. sp. k. (Poland)

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1. General comments – overview

Stakeholder no. *(See cover page)*	General comment (if any)	Outcome (if applicable)
1	We generally support the idea that a standardized presentation will facilitate the evaluation process however we have reservation as to the possibility that this will create redundant sections in the CTD. In addition, we see a need for alignment between this Appendix IV and the current Volume 2B.	The purpose of Appendix IV is to provide a standardised format to summarize data. The inclusion into the CTD is intended in Module 2.7.1 where such summaries are already presented hence this would not lead to redundancy.
1	The EGA would appreciate if the EMA could clarify in the final Appendix IV what are the exact expectations in cases of BCS biowaivers (Class 1 and 3). It is particularly relevant to address the likely redundance of this section 2.7.1 with others such a 5.3.1.2.	The template tables of Appendix IV were primarily designed for generic submissions based on bioequivalence trials, and are not necessarily suitable for BCS-based biowaiver submissions. At present, a similar template for presentation of data from BCS-based biowaiver submissions in Module 2.7.1 does not seem to be necessary. Of note, source documents like dissolution reports should in general not be part of Module 2. In Module 2.7.1 only a summary should be given and source documents should be included in Module 5.
1	In addition, in the cases of BCS biowaivers, clarifications would be needed as to the requirements to fill in the template tables in situations where the medicine dissolves in 15 minutes.	It is not expected that these template tables can cover every situation. In this specific case, the standardization of the format would not offer any advantage as the presentation of the data appears straightforward.
1	According to the Questions and Answers Document of European Commission on the rules governing medicinal products in the European Union, "Volume 2B Presentation and content of the dossier Common Technical Document (CTD) 2003 Edition updated on February 2008; presentation of Clinical Summaries (Module 2.7.1) for	The template tables refer to the data requirement as identified in the Guideline on the Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98/rev. 1), and the Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009). The objective of this

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	the generic and hybrid applications is not mandatory. "Question 8: Generic and hybrid applications For applications according to Article 10(1) of Directive 2001/83/EC (so called "generic applications") and for applications according to Article 10(3) of Directive 2001/83/EC (so called "hybrid applications"), what should Module 2 contain? Answer: For applications according to Article 10(1) and (3) of Directive 2001/83/EC, Module 2 must include : - Quality Overall Summary - Non-clinical Overview - Clinical Overview Non-clinical and Clinical Summaries can be provided, but they are only mandatory if new additional studies have been provided within the documentation. The written summary of the bioequivalence has to be part of the Clinical Overview." Therefore, it is our understanding that under the current legal interpretation, generic of hybrid applications can present the biopharmaceutical and bioanalytical summary tables only in the Module 2.5 Clinical Overview, or Module 5.2 Tabular Listing of All Clinical Studies of the CTD Dossier. For consistency purposes, the EGA would request the EMA to - either introduce a statement in Appendix IV which allows companies to present biopharmaceutical and bioanalytical	Appendix to the Guideline on the Investigation of Bioequivalence is to assist applicants and facilitate the evaluation process likewise, particularly for generic applications according to Directive 2001/83/EC, Article 10(1), but also for other applications including variations, fixed combinations, extensions and hybrid applications. It is recommended that the summary tables should be provided in Module 2.7.1. The referred to Question and Answer document is acknowledged. According to current regulatory experience however Module 2.7.1 is usually provided which is more in line with the recommendations of the Notice to Applicants Volume 2B. The location of the suggested template tables in Module 2.7.1 is therefore understood to represent current regulatory practice and recommendation.

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	data summary tables in Module 2.5 or Module 5.2, referencing to Volume 2B or, indicate clearly that a change of Volume 2B in this regard is being considered.	
2	Proposed change: In the Appendix "Plasma" should be replaced by "biological matrix" to make it more generically applicable.	Agreed. Table 4 has been changed accordingly.
3	The document does not clearly state where exactly to put the described tables. We would suggest placing this kind of tables in the Appendix 4 of the 2.7.1 document.	Agreed. Changes in the text are however not necessary as the introduction already states that " This Appendix contains a set of template forms to assist applicants in the preparation of Module 2.7.1 ..."
5	Please consider to replace "plasma" by "sample matrix" throughout the whole document as "sample matrix" is a more broader term and encompasses for example also blood, serum, urine or whatever matrix applicable.	Agreed. Table 4 has been changed accordingly and the term "biological matrix" was used instead of "sample matrix."
7	We appreciate the efforts to standardise the presentation of data in CTD Module 2.7.1 and the tables provide helpful guidance. Format and contents of the CTD have been defined globally, under the umbrella of ICH. We therefore interpret this document as a way to facilitate preparation of 2.7.1 and not to provide a mandatory format (we acknowledge that use of the tables is encouraged). It would be helpful, however, if the non-mandatory nature could be stated explicitly while making a reference to ICH for background.	This is already expressed in the Introduction, and further clarified in the revised text. Please also refer to the comment above regarding the Question and Answer document.
9	BCS Class III Biowaiver: Within the guidance CPMP/EWP/QWP/1401/98 Rev.1/Corr** in Appendix III section IV.2 it states "If a biowaiver is applied for a	The proposal is out of the scope of this Appendix which provides template tables for data presentation only but is not intended to provide clarifications on the scientific

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	BCS-class III drug substance excipients have to be qualitatively the same and quantitatively very similar in order to exclude different effects on membrane transporters". With product reverse engineering techniques, we get the approximate quantities of excipients used within the formulation of the product. For well established excipients with known effects (if any) on membrane transporters, gut transit time etc the important fact is not the qualitative and quantitative similarity with the reference product but an Expert Assessment of the clinical significance of any differences that may or may not affect bioavailability. We propose that advantage is taken of this opportunity to clarify this aspect of the guideline by making this change. This will make the guideline much more useful in promoting the availability of affordable quality medicines.	content of the guideline.
9	Impact on Harm reduction: Within the guidance CPMP/EWP/QWP/1401/98 Rev.1/Corr** in section 4.1.3 it states "In order to reduce variability not related to differences between products, the studies should normally be performed in healthy volunteers unless the drug carries safety concerns that make this unethical". We consider additive substances like nicotine should fall under this principle and that this point should be clarified in the guideline as soon as possible We have had discussions with Health Authorities regarding nicotine bioequivalence studies where we have had conflicting advice that the studies should contain non-smokers as well as smokers.	The proposal is out of the scope of this Appendix which provides template tables for data presentation only but is not intended to provide clarifications on the scientific content of the guideline.

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	Exposing non-smokers (i.e. not the target population) to an addictive substance such as nicotine in a bioequivalence study intended to support a MAA indicated only for use in nicotine addicts (i.e. smokers, the target population) is scientifically pointless and ethically unsound – never the less this is what some Competent Authorities are asking us to do. It was asked due to the fact that majority of the subjects had pre-dose levels equal to or greater than 5% of Cmax. We recommend that the guidance clarifies that for substances such as replacement nicotine used to reduce or replace dependence on cancerous sources of nicotine, i.e. from cigarette smoking, that the guideline with regards to 5% of Cmax be put into context as the target population will inevitable have a residual Cmax and that therefore it should be controlled for and not simple eliminated as this will cause problems in subject recruitment. Therefore baseline corrections are acceptable provided study has a method to detect contamination (in the case of nicotine, CO monitoring before dosing).	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Lines 25-27	2	"...it is anticipated that a standardized presentation will facilitate the evaluation process. Applicants are therefore encouraged to use these template tables when preparing Module 2.7.1." Is there an <u>expectation</u> that applicants who are in the process of compiling their application dossiers and nearing MAA submission should present biopharmaceutic data using the tables in the draft Appendix? There is a concern that if there is such an expectation, an application that does not follow Appendix IV will be deemed invalid after the Appendix comes into effect; or if an MAA has been locked down in close proximity to the date of the Appendix coming into effect, this would not allow for adequate time to alter the presentation of data to be in line with the Appendix. <u>Proposed change:</u> "...it is anticipated that a standardized presentation will facilitate the evaluation process. ~~Applicants are therefore encouraged to use these template tables when preparing Module 2.7.1.~~" Applicants are therefore encouraged to use these template tables when preparing Module 2.7.1 but are not required to do so until the Appendix comes into effect. A reasonable judgement will be permitted for applicants who have submitted an MAA in close proximity to the	Not accepted. Once this Appendix IV to the Guideline on Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98/rev. 1) has been adopted by CHMP it will be published for 6 months before coming into operation. Recommendations of the Appendix IV should be applied to all applications submitted after the Appendix has come into operation, regardless of when the BE studies were conducted. See also EMEA/P/24143/2004.

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		date of the Appendix coming into effect, such that if the presentation of data does not follow the template tables, the application will not be deemed invalid even if the Appendix comes into force soon before the date of submission of the application	
Lines 28-29	2	Comments: The requested tables are reasonable for generic applications in Europe, but may represent increased difficulty for innovator applications as in these cases Module 2.7.1. is written for submission in multiple regions in the world Proposed change: Please clarify if the statement "might be used in other applications" applies to innovator applications.	Accepted. The Introduction part now more clearly defines the scope of this Appendix: "This Appendix is intended for generic applications according to Directive 2001/83/EC, Article 10(1). But if it is applicable then it is also recommended to be used in other applications such as variations, fixed combinations, extensions and hybrid applications." "Innovator" applications (Article 8(3) applications) are not among the listed application types.
Line 42	2	Comments: Typo? "applicants are encouraged using..." Proposed change: "are encouraged to use..."	Accepted.
Lines 47 - 48	2	Comments: If the tables are searchable and not scanned is a WORD or RTF document for Module 2.7.1 mandatory? Proposed change: Encouraged to provide Module 2.7.1 also in Word	Not accepted. The term "other standard searchable text format is too general. It might include proprietary document formats such as docx or wpd or public document formats as xml or html. Working with these data formats might need special software which is not available for the assessors. Archiving and

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		(.doc), RTF, or other standard searchable format	retrieving data would potentially be another problem.
Lines 50-52	2	Comments: Perhaps it would be helpful to reference the quality section "Module 3.2.P.2.2.3 Physicochemical and biological properties of the drug product" for BioPharm discussion on drug substance physico-chemical properties and drug product dissolution performance to support bioequivalence studies and biowaivers. Proposed change: Add a reference the quality section "Module 3.2.P.2.2.3 Physicochemical and biological properties of the drug product".	Not accepted. Possibly several other references will be made in the Summary of Biowaiver Justification and Module 3.2.P.2.2.3 is only one of them. There is no reason to give more emphasis to this Module than to any other.
Lines 50-51	10	Comment: It is stated that relevant data for justification of BCS-based biowaiver requests should be included in Module 5.3.1.2 "Comparative BA and Bioequivalence (BE) Study Reports". Does it mean that full justification of BCS-based biowaiver should be presented in section 5.3.1.2 or "row data", e.g. the report from dissolution tests?	Accepted. Yes, relevant source data as the dissolution reports should be presented in Module 5.3.1.2.
Table 1.1	2	Comments: "%" addresses each ingredient expressed as a percentage of the total core or weight. Proposed change: "%" should also address w/v % (for solutions). This	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		should be specified in the explanation given under the table.	
Table 1.1	10	Comment: It is not clear whether data for BCS-based biowaiver of all strengths of an applied medicinal product should be presented in Module 2.7.1. In table 1.1 the MAA should include information concerning qualitative and quantitative composition of the test product. In case of BCS-based biowaiver of all strengths of a applied medicinal product a new table should be added that would present information on at least qualitative composition of the reference product. According to the Appendix III of the guideline CPMP/EWP/QWP/1401/98 Rev. 1/ Corr** the usage of the same excipients in similar amounts in the test product as in the reference product is advisable for BCS-based biowaiver. Proposed change: In Module 2.7.1 the MAA should include relevant data for justification of BCS-based biowaiver of all strengths of a applied medicinal product.	Accepted The MAA should include all relevant data for justification of BCS-based biowaiver request in Module 2.7.1. However, the source data should be presented in Module 5.3.1.2.
Table 1.2	2	Comment: We believe that F2 has limitations that can cause bioequivalent products not to be deemed equivalent in vitro. Proposed change: It would be helpful to add the statement from the	Partly accepted. The instructions to table 1.2 were modified to cover the case when f2 could not be used.

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		guideline to the instructions: "Alternative methods to the $f2$ statistic to demonstrate dissolution similarity are considered acceptable, if statistically valid and satisfactorily justified	
Table 1.2	2	Comment: According to the Bioequivalence guideline the evaluation of the similarity factor is based on a relative standard deviation of mean percent dissolved to be less than 20% for the first point and less than 10% from the second to last time point. Proposed change: Addition of the relative standard deviation to the mean values of percent dissolved in function of time.	Partly accepted The following sentence was added to the instructions: "Only the mean percent dissolution values should be reported but denote the mean by star (*) if the corresponding RSD is higher than 10% except the first point where the limit is 20%"
Table 1.2	2	Comments: 1. "Average Percent of Label" should be presented as a column header for the results 2. Temperature should be added as a dissolution condition 3. Instructions to this table indicate that a similar table is required for reference product if "sink condition" could not be achieved. This should be clarified. Perhaps this means if average dissolution is < X% at the final timepoint. The actual threshold for sink should be clearly described.	Partly accepted. The definition of sink conditions is out of the scope of this Appendix. The instruction to provide additional dissolution data if the sink conditions were not achieved led to misunderstandings and it was removed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: 1. Please add "amount of dissolved active ingredient specified in the individual monograph, expressed as a percentage of the labeled content" to instructions and "Average Percent of Label" should be presented as a column header for the results 2. Add temperature to table description 3. Please add to instructions: "sink conditions are considered to exist if, at the dissolution of 100% of the highest strength of the product to be tested, a concentration of not more than 1/3 of saturation will be achieved."	
Page 5, Table 1.2, Text below table, last sentence	2,3	Comment: Please clarify the meaning of the last sentence on this page as it is unclear which data should be provided if sink conditions could not be achieved	The instruction was removed.
Page 5, Table 1.2, Text below table, last sentence	10	Comment: Under the Table 1.2 *In vitro dissolution data for biowaiver request*, it is stated that a similar table for the reference product should be filled if sink condition could not be achieved. Does it mean that in this table, the results of dissolution tests for additional strength(s) of the reference product should be presented? Relative to which strength and product should f2 values be	The instruction was removed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		computed?	
Table 2.1	2	Comment: We are unsure to what "Assay%" refers– is it equivalent to "Accuracy" (FDA terminology)? Proposed change: Please clarify "Assay%"	Accepted. The terminology has been changed to "Measured content (% of label claim) "
Table 2.1	2	Comments: *In vitro* dissolution profiles are performed with the test and reference products that will be used in the in vivo study. Proposed change: Addition of the dissolution profiles of test and reference products (and strength) that are used in the bioequivalence study.	Not accepted. This part should be discussed in Module 2.3
Table 2.1	2	Comment: The term "product certificate" is used (second to last row). This term should be clarified as products can get several different types of certifications. I believe the intended certificate is a "certificate of analysis". Proposed change: Suggest replacing "product certificate" by "certificate of analysis	Accepted.
Table 2.1	2	Comment: There is a footnote mark "1" in the "Manufacturer" row	Accepted. The footnote mark was a typographical error and has been

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		with no corresponding footnote. Proposed change: Add a footnote or remove the mark in the table.	removed.
Table 2.1	10	Comment: In Table 2.1 *Test and reference product information*, there is a footnote with number 1 concerning the manufacturer, however, no information has been presented under this footnote. Is there any specific requirement for the information on the manufacturer that should be presented in this table?	Accepted. The footnote mark was a typographical error and has been removed.
Table 2.1	7	Comment: The footnote 1 is not explained. Proposed change: Explanation should be added.	Accepted. The footnote mark was a typographical error and has been removed.
Table 2.2 Table 2.3	2	Comment: Please clarify that the proposal for the table layout is trial by trial. That is, would the EMA like a separate table for each trial, rather than concatenation of multiple trials into the same table? Proposed change: In the case where there are multiple studies, we suggest that rather than having specific items listed in rows – they should be in columns. This will facilitate overview across trials	Partly accepted. It has been clarified that separate Table 2.2 and Table 2.3 are needed for each trial.

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Table 2.2	2	Comment: In Table 2.2, the Agency is asking for inspection dates of an EU authority inspection. However, section 2.7.1 is part of the CTD and information should be applicable to any country, in order to avoid rewriting of documents for each country where the CTD is submitted. Proposed change: We suggest deleting the following column "Has this site been inspected by an EU Authority?". Or perhaps substitute "Has the site been inspected by an EU Authority" by "Has the site been inspected by a Health Authority?"	Not accepted. The scope of this Appendix is to provide recommendations for such template tables from an EU perspective.
Table 2.2	10	Comment: To facilitate data entry and reading of the table some changes seem to be necessary. Proposed change: ~~Clinical Study Site~~ => Clinical Site ~~Bioanalytical Facility~~ => Bioanalytical Site ~~Study period~~ => Dates Name / Address / Dates / Authority & Year => Rather in separate rows than in columns ~~Has this site been inspected by an EU Authority?~~ => Last inspection by EU Authority	Partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Table 2.3	2	Comment: Volunteers typically describes a healthy population and may not be perceived as covering healthy as well as patients. Proposed change: Change # Volunteers to #subjects.	Accepted.
Table 2.3	6	Comment: For "#Volunteers (evaluated)", it should be made clear whether this refers to the number of volunteers evaluated for PK/statistical analysis or for safety. Proposed change: #Volunteers (PK/statistical analysis)" and/or #Volunteers (safety evaluation)"	Accepted
Table 2.3	10	Proposed change: ~~Fasting, Fed~~ => Fasting/ Fed Dose: / Single/Multiple dose: / Number of periods: / Two-stage design: (yes/no) / Fasting, Fed: / # Volunteers (dosed): / # Volunteers (evaluated): => Rather in columns than in rows.	Partly accepted. Some of the recommendations were accepted.
Table 3.1	2	Comment: Arithmetic Mean and Standard Deviation are used in this table. However, since AUC, Cmax and Tmax and typically not normally distributed, it is more standard to express pharmacokinetic parameters in geometric	Accepted.

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		mean with a coefficient of variation. Proposed change: Please add that Geometric Mean (CV%) may be substituted for Arithmetic Means (±SD)	
Table 3.1	4	Comment: Use tmax instead of Tmax in order to keep consistency with the GL. AUC(0 t), AUC(0 ☐), and Cmax are generally assumed to follow a lognormal distribution, whereas tmax a discrete distribution. Arithmetic means (±SD) therefore are statistical not justified and generally not included in the study report. Even if these parameters are given in the report for informational purposes, bioequivalence is calculated as the ratio of geometric least squares means. Proposed change: Geometric least squares means (GLSM) ±SD (alternatively geometric CV) for AUC(0 t), AUC(0 ☐), and Cmax. Median (quartiles) for tmax.	Partly accepted. Arithmetic Means (±SD) may be substituted by Geometric Mean (±CV%). A footnote was added to allow this option.
Table 3.1	8	Comment: We note the request to present tmax data in the form of arithmetic means and standard deviations. As a non-continuous parameter, we have always summarised such data in the form of medians and ranges.	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: We would like to propose the presentation of: • tmax data in the form of medians and ranges as an acceptable alternative to arithmetic means and standard deviations. • geometric mean for AUC and Cmax instead of arithmetic mean. • log SD for AUC and Cmax instead of SD.	
	2	Comments: Footnote is missing for "**" What is the intention of this footnote? Is it needed at all? It is specified that arithmetic mean is used for Tmax - median is often more appropriate. Proposed change: We suggest that the following be included in the footnote "**"(currently missing) "median (max;min) may be used for Tmax, if appropriate"	Accepted.
Table 3.1	2,3	Comment**:** Please clarify what is meant with "others" (parameters) Proposed change: We believe it is sufficient to display the respective AUC, Cmax and Tmax value. There is no need to give any further PK parameter in addition in this table.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Please remove "others".	
Table 3.2	2	Comment: The use of the term "carry-over" is confusing as it has different meanings for PK scientists and bioanalytical scientists. Proposed change: We suggest the following : "Records with pre-dose sample > 5% Cmax	Accepted.
Table 3.3	2,3	Comment: Please clarify what is meant with "ratio" Proposed change: We assume that the geometric mean ratio is being meant in this table, since this is being normally be calculated within an ANOVA:	Accepted.
Table 4.1	2,7,10	Comment: Reference to footnote 1 could not be found in the Table. Please confirm it is necessary.	Accepted. The missing footnote marks have been added
Table 4.1	2	Comment: Not all standard curves are linear. For that reason, linearity should not be an expectation. Proposed change: We propose changing "Linearity" to "Regression Fit" and change "R" to "R2". Alternatively, to cover for the situation of assays with non linear regression, replace	Partly accepted. The table entry has been deleted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		the row for linearity with "Calibration model and weighting factor".	
Table 4.1	2	Comment: Matrix factor is not always calculated or is not performed with some MS/MS methods. Proposed change: Please add "when relevant"	Accepted.
Table 4.1	2	Comment: Different laboratories calculate the matrix factor differently so it may be hard to interpret the value. Proposed change: We suggest asking the laboratories to add the formula they used so the value can be appropriately interpreted.	Not accepted. The MF should be calculated as described in the Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009).
Table 4.1	2	Comment: "Short term stability of the stock solution and working solution": Proposed change: This should be optional and mentioned only if no long term stability on stock and working solution are available	Accepted.
Table 4.1	2	Comment: Short term stability in plasma at RT: when "observed change" is mentioned: if acceptance criteria are	Partly accepted. This Appendix does not specify any acceptance criterion.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		fulfilled than "Observed change is not relevant". Does this mean observed change beyond acceptance criterias are accepted? Or does this relate to accuracy and CV%? Proposed change: Please clarify the necessity of this portion of the table as it does not seem to be present in other guidelines	The Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009) requests to report the results of short term stability studies at RF or at sample processing temperature.
Table 4.1	2	Comment: "Post preparative stability" Proposed change: should be put after "Autosampler stability"	Accepted.
Table 4.1	2	Comment: Post-preparative stability is not necessarily conducted on a dry extract, so the template should allow enough flexibility. Proposed change: We suggest the following rewording: "Post-preparative stability (dry extract stability list condition)"	Partly accepted. The text in parenthesis has been deleted.
Table 4.1	2	Comment: Long term stability in plasma (vol/page,link): Proposed change: is this about validation QC stability? Would put this just after Stab at RT	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Table 4.1	2	Comment: "freeze and thaw stability": Proposed change: needs re-ordering this after long term stab	Accepted.
Table 4.1	2,3	Comment: Partial validation 2: not sure to understand what results need mentioning as a result of the re-validation? e.g. re-validation would imply possibly at minima an Intra LLOQ exercise Similarly do we need to mention the result of the cross validation and how (QC, CV and accuracy?) Proposed change: needs clarifications	Accepted
Table 4.1	2	Comment: it is not clear whether the footnote refer to the partial validation because there is partial validations 1 and partial validations 2	Accepted.
Table 4.1	2	Comment: some terms should be corrected Proposed change: LLOQ, r^2 Footnote [1] should be added to following issues: short term stability of the stock solution and working	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		solution, long term stability of working solutions and post-preparative stability.	
Table 4.1	2	Comment: There is no description of the assay performance at LLOQ or ULOQ in terms of matrix effect. Proposed change: Addition of a line with "Matrix effect at LLOQ and ULOQ" in the first column with <Accuracy, Precision> in the second column.	Partly accepted. In this row, the table entries are the regulatory benchmark criteria set by the Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009).
Table 4.1	2	Comment: For the quantitation of Biotherapeutics, it is important to evaluate the effect of the presence of Anti-Drug Antibody on the performance of the assay to quantify the drug. Proposed change: Addition of a line with "Effect of ADA" in the first column and <Maximum Tolerated Concentration evaluated with positive control> in the second column.	Not accepted. Biologicals are outside the scope of this Appendix.
Table 4.1	2	Comment: Table is only suitable for Chromatographic assays and not Ligand Binding assays Proposed change: Suggest to emphasise that table is applicable for both types of methods and that specific issues for where a	Accepted.

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		parameter is not relevant can be noted "NA"	
Table 4.1	4	Comment: Short-term stability in plasma at room temperature (QC). Proposed change: Change 'plasma' to 'biological matrix' in order to keep consistency with the drafted GL on validation of bioanalytical methods (EMEA/CHMP/EWP/192217/2009).	Accepted.
Table 4.1	4	Comment: 'Linearity' is not applicable to many bioanalytical methods. Quadratic calibration is commonly applied if a wide range of concentrations is covered (e.g. MS-methods), quenching occurs (fluorescence), or is nonlinear (4- and 5-parameter logistic models in ligand binding assays). Linearity is not mentioned in EMEA/CHMP/EWP/192217/2009 at all; the GL mentions only 'calibration curve' [sic!]. 'r' (coefficient of correlation – applicable to linear functions only!) is meaning¬less, unless the calibration function, the number and location of calibrations, and the weighting function is given for all (!) valid batches as well. Proposed change: Delete.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Table 4.1	4	Comment: Type of biological matrix (e.g. blood, plasma, urine, saliva or tissue) and type of anticoagulant should be requested in order to keep consistency with EMEA/CHMP/EWP/192217/2009. Proposed change: Add.	Partly accepted. Saliva or tissue are not relevant biological matrixes in the context of this Appendix. Anticoagulant is an important detail but no regulatory decision is based on that.
Table 4.1	5	Comment: Difference between autosampler stability and post-preparative stability (dry extract stability) not clear – please specify.	Accepted According to the Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009) post-preparative stability defined as "stability of the processed sample at room temperature or under the storage conditions to be used during the study (dry extract or in the injection phase)" while autosampler stability defined as " on-instrument/ autosampler stability of the processed sample at injector or autosampler temperature"
Table 4.1	5,6	It is not clear whether footnote "1" is referring to "Partial validations 1" or something else Footnote 1 is mentioned at the bottom of the table, but it is not clear what it refers to.	Accepted. The text has been changed to clarify the intentions.
Table 4.1	5	Row 9, standard curve concentrations: units would be more universal than units/mL as concentrations may also be given in e.g. nmol/L	Accepted.
Table 4.1	6	Comment: It is requested to give the Matrix Factor%. As the	Not accepted. The final version of the Guideline on Bioanalytical Method

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		bioanalytical guideline is not yet finalised, it is suggested not yet to request this metric. Proposed change: Please delete Matrix Factor% from the table.	Validation (EMEA/CHMP/EWP/192217/2009) is adequately reflected in the updated template tables.
Table 4.2:	2	Comment: Longest storage period should be clarified. Is this table only required for pivotal trials. If yes, the corresponding footnote is missing.	Accepted.
Table 4.2	2	Comment: More information, e.g. temperature, should be asked in this table	Accepted.
Table 4.3	2	Comment: The following should be changed to reflect differences in assay methodology and with the EMA guideline for assay validation: Percentage of samples where the difference between the two values was less than 20% of the mean" Proposed change: Percentage of samples where the difference between the two values was less than 20% of the mean for LC-MS assays and less than 30% for ligand binding assays	Accepted.
	5	Comment: 20% difference does only apply to small	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		molecule/chromatographic assays Proposed change (if any): Please consider to use the following phrase "Percentage of samples where the difference between the two values was less than 20% of the original value for chromatography based assays (small molecules) and less than 30% for ligand binding assays".	
Table 4.3	2	Comment: Total number of valid analytical runs is supposed to not include incurred sample reanalysis runs. This does not allow the analysis of study samples and ISR samples in the same run. Proposed change: [1] Without incurred sample reanalysis	Not accepted. It is agreed that total number of valid analytical runs is supposed to not include incurred sample reanalysis runs. The footnote intends to highlight this restriction.