14th EBF Open Symposium

Science – Our Universal Language

EBF feedback to industry and FDA on FDA Bioanalytical Method Template

Luca Ferrari and Tom Verhaeghe, on behalf of the EBF

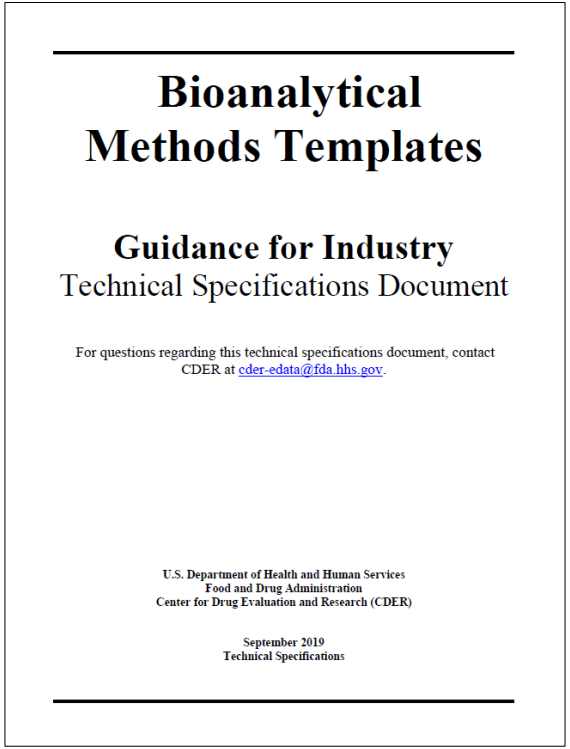
25 November 2021, Barcelona

Background information

In Sept 2019 the FDA published a Guidance for Industry.

It contains ready to use templates to submit BA method summaries used in clinical pharmacology studies for NDAs/BLAs, as part of the Common Technical Document (CTD) for the Registration of Pharmaceuticals for Human Use.

A FotP survey was organized in 2020 to collect feedback on the interpretation and willingness to adopt this guidance.



Bioanalytical Methods Templates

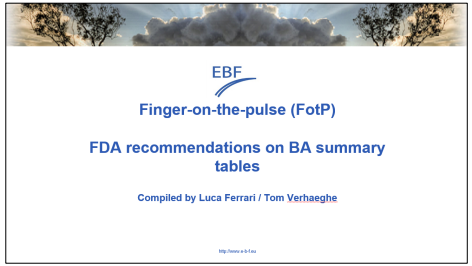
Guidance for Industry
Technical Specifications Document

For questions regarding this technical specifications document, contact CDER at cder-edata@fda.hhs.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

September 2019
Technical Specifications

Content of the FotP



Nine questions were selected to investigate whether:

- this guidance had been adopted within the EBF community
- comments had already been received for NDAs/BLAs
- this technical document was considered to be making other filings difficult and conflicting with ICH M10
- this topic should have been further discussed at the EBF

35 responses were received

Feedback by most responders highlighted:

- Willingness to fully implement the guideline in the future
- Desire to have this topic further discussed at the EBF, in particular to have some clarity/agreement on how to compile the tables

The team

A team was established in October 2020:

Luca Ferrari	(Roche)	co-chairs
Tom Verhaeghe	(Janssen)	
Lene Andersen	(Orphazyme)	
Elke Zwanziger	(Roche)	
Eva Dam Christoffersen	(AscendisPharma)	
Tobias Haslberger	(Abbvie)	
Berthold Lausecker	(Az-Biopharm)	

9 TCs were organized

Objectives of the Team

- Review the FotP survey results, collect feedback on experience gained in recent filings
- Thorough evaluation of the technical requirements defined in the guideline

Action plan:

1. To generate an example document: tables populated with real study data, including validations and clinical BA studies
2. To prepare a guide providing information on the way the summary tables should be compiled

Example tables

- The team decided to include the following data:
  - two methods/validations
  - two clinical studies
  - one cross validation
  - 2 analytes for CC, 1 analyte for LBA assays

- Both Chrom & Ligand Binding Assays were considered

Further steps – the extended team

- The preliminary outcome was presented at the ‘All members meeting’ in April 2021
- It was decided that example tables and guide would be pressure tested by a second round of review including a larger group of EBF representatives from 17 companies

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Tom Verhaeghe, Janssen
Lene Andersen, Orphazyme
Elke Zwanziger, Roche
Eva Dam Christoffersen, AscendisPharma
Tobias Haslberger, Abbvie
Berthold Lausecker, Az-Biopharm

Benno Ingelse, Byondis
Matti Kinberg, Synexa
Rob Nelson, Covance
Martin Rieger, Formycon
Jean Mark Gnoth, Bayer
Marianne Fjording, Bioagilytix
Gwenda Pynaert, Argenx
Klaus Pusecker, Merck
Sirpa Laakso, Orion
Nico van de Merbel, PRA

Table 1: Bioanalytical Method Life Cycle Information

	Method validation #1	Method validation #2	Study#1	Study#2
Analyte	Drug A	Drug A + Metabolite M	Clinical Study XY	Clinical Study YZ
Validation type	Full validation	Full validation + cross-validation with Method validation #1	In-study	In-study
eCTD reference number	Add number	Add number	Add number	Add number
Method ID	Method A	Method B	Method A	Method B
Duration of time method is in use	2011-	2014-	Jan 2012 - Dec 2013	Jan 2014 – Mar 2014
Bioanalytical site	Company name	CRO name	Company name	CRO name
Matrix	Human EDTA plasma			
Platform	CCs: Liquid chromatography/mass spectrometry (LC-MS/MS) LBAs: Meso Scale Discovery Electrochemiluminescence (MSD ECL)			
Format	CCs: Protein precipitation & LC-MS/MS LBAs: Bridging immunoassay, capture via biotinylated specific antibody against drug A, detection via Sulfo-Tag™ labelled antibody against drug A			
Stock reference, lot number, expiration date	Drug A; lot n. XY; expiry date 30 Jun 2012 lot. n. YZ; expiry date 30 Nov 2015	Drug A; lot. n. YZ; expiry date 30 Nov 2015 Metabolite M: Lot.n. WX; retest date 02 Feb 2017	Drug A; lot n. XY; expiry date 30 Jun 2012	Drug A; lot. n. YZ; expiry date 30 Nov 2015 Metabolite M: Lot.n. WX; retest date 02 Feb 2017
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	1.00 ng/mL to 2000 ng/mL	Drug A: 1.00 ng/mL to 2000 ng/mL Metabolite M: 1.00 ng/mL to 2000 ng/mL	1.00 ng/mL to 2000 ng/mL	Drug A:1.00 ng/mL to 2000 ng/mL Metabolite M: 1.00 ng/mL to 2000 ng/mL
Matrix study population	Healthy	Healthy and Rheumatoid Arthritis	Healthy	Rheumatoid Arthritis

CCs: Chromatographic Assays
LBAs: Ligand Binding Assays

Table 2a: method performance (in validation)

For each '*'add additional products as needed; additional products are usually more applicable for 351(k) products

Bioanalytical method validation report name, amendments, and hyperlinks	Method A validation report name, amendments and hyperlinks		
Method description	CCs: (Analytical procedure for the Determination of Drug A in Human Plasma using) Protein Precipitation followed by Liquid Chromatography with Tandem mass Spectrometric Detection (LC-MS/MS) using a stable isotope labelled/structural analog internal standard. LBAs: Serum Bridging electrochemiluminescence (ECL) immunoassay, capture via biotinylated specific antibody against drug A, detection via Sulfo-TagTM labelled antibody against drug A		
Materials used for standard calibration curve and concentration	CCs: Blank human EDTA plasma; 1.00-2.00-5.00-10.0-20.0-50.0-100-200-500-1000-2000 ng/mL LBAs: Blank human serum; 0.05*-1.00-2.00-5.00-10.0-20.0-50.0-100-200-500-1000-2000-4000* ng/mL (*anchor calibrators)		
Validated assay range	1.00-2000 ng/ml		
Material used for quality controls (QCs) and concentration	Blank human EDTA plasma; 1.00 (LLOQ)-2.80 (Low) -60.0 (Medium)-1560 (High)-15600 (Dilution #1)-154000 (Dilution #2) ng/mL		
Minimum required dilution (MRD)	CCs: Not Applicable LBAs: 1:100		
Source and lot of reagents	CCs: Not Applicable LBAs: Biotinylated antibody A Source: Company X; Sulfo-tagged antibody B Source: Company Y		
Regression model and weighting	CCs: 1/x2 weighted Linear regression LBAs: 5PL Marquardt with $1/Y^2$ weighting		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ (calibration line in singlicate)	11	Table X in Report Y
	Cumulative accuracy (%bias) from LLOQ to ULOQ Product A *	-4.0% to +3.0%	Table X in Report Y
	Cumulative precision (%CV) from LLOQ to ULOQ Product A *	≤ 2.0%	Table X in Report Y able 2 of Method A Validation report
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs** QCs for product A: Please list *	+1.9% to +7.7%	Table X in Report Y
	Inter-batch %CV QCs for Product A: Please list *	≤ 3.6%	Table X in Report Y
	Total Error (TE) QCs for Product A: Please list *	CCs: NA LBAs: ≤ 21.3%	Table X in Report Y

CCs: Chromatographic Assays
LBAs: Ligand Binding Assays

Selectivity & matrix effect	CCs: 6 lots tested, Bias: -9.6% to +1.0% selectivity Bias: -10.6% to +3.5% matrix effect LBAs: 9 out of 10 healthy serum samples: %bias (–9.3) to 11.0% at LLOQ; 1 serum sample failed the 25% Bias criterion at LLOQ. 9 out of 10 healthy serum samples: %bias (–15.6) to (7.0)% at high QC. 1 serum sample failed the 20% Bias criterion at high QC.	Table X in Report Y
Interference & specificity	CCs: 6 lots tested. No interference at RT of analyte or IS. Drugs tested: Drug A: no interference Drug B: no interference Drug C: no interference… Interference with Drug A: the blank samples spiked with Dug X at 3300 ng/mL did not contain any peaks at the retention time of Drug A (>20.0% of the LLOQ calibration standard response) or ISTD (>5.0% of the ISTD peak response in the control zero sample). %Bias at LQC: -6.7 to -4.3 Interference for Drug B:…. Interference for Drug C:… LBAs: e.g. describe interference with target: e.g.Structurally related Drugs tested for selectivity: Drug A: no interference Drug B: no interference Drug C: no interference… e.g. describe interference with Anti-Drug Antibodies (ADA)	Table X in Report Y Table X in report Y
Hemolysis effect	One lot tested. %Bias: -8.4 to -7.3	Table X in Report Y
Lipemic effect	One lot tested. %Bias: -3.3 to 5.5	Table X in Report Y
Dilution linearity & hook effect	Dilution Linearity: Highest concentration tested: X ng/mL Range of dilutions tested: 1/10, 1/100, 1/1000, 1/5000 Highest dilution tested: 1:X Cumulative %bias: –8.6% to 10.0% Hook Effect (LM): A hook effect was not observed for the concentrations tested (include highest concentration tested)	Table X in Report Y
Bench-top/process stability	Blood: 4h on melting ice; 4h at room temperature Plasma: 72h at room temperature Processed samples: 96h in autosampler at room temperature	Table X in Report Y
Freeze-Thaw stability	4 F/T cycles at -20°C/room temperature	Table X in Report Y

CCs: Chromatographic Assays
LBAs: Ligand Binding Assays

Table 2a: method performance (in study)

Method performance in study#1		
Assay passing rate	100% (7/7)	Table X in Report Y
Standard curve performance	• Cumulative bias range: -1.5% to +2.0% • Cumulative precision: ≤ 3.4% CV	Table X in Report Y
QC performance	• Cumulative bias range: -3.7% to +0.7% • Cumulative precision: ≤ 4.0% CV • TE: Not Applicable (SM), ≤ 18.0% (LM)	Table X in Report Y
Method reproducibility	Incurred sample re-analysis was performed in 48/232 (21%) of study samples, and 91.7% of the samples met the pre-specified criteria.	Table X in Report Y
Parallelism	Incurred sample parallelism was performed in 10/232 study samples, and 100% of the samples met the pre-specified criteria. Or: Parallelism was not performed for this study	Table X in Report Y
Study sample analysis/ stability	Maximum frozen storage for STDs/QCs and study samples was 317 days. Samples and STDS/QCs were analyzed within proven frozen stability of 326 days at -20°C.	Table X in Report Y
Standard calibration curve performance during accuracy and precision runs	CCs: 11; 1.00-2.00-5.00-10.0-20.0-50.0-100-200-500-1000-2000 ng/ml LBAs: 11; 0.05*-1.00-2.00-5.00-10.0-20.0-50.0-100-200-500-1000-2000-4000* ng/ml *anchor calibrators	

CCs: Chromatographic Assays
LBAs: Ligand Binding Assays

Table 2b: method modifications and cross-validations

Bioanalytical method validation report name and hyperlink	Method A and Method B Validation report name		
Changes in method	Transfer to another lab		
New validated assay range if any	Assay range not changed		
Validation parameters	**Cross-validation performance**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Cumulative accuracy (% bias) in standard calibrators from LLOQ to ULOQ		see data in validation reports for method A and method B
	Cumulative precision (% CV) from LLOQ to ULOQ		see data in validation reports for method A and method B
Performance of QCs during accuracy and precision runs	Cumulative accuracy (% bias) in 5 QCs		see data in validation reports for method A and method B
	Inter-batch % CV		see data in validation reports for method A and method B
	Percent TE		see data in validation reports for method A and method B
Cross-validation	18 spiked QCs; low-mid-high QCs in 6 replicates Method A: bias +1.3% to +3.6%; CV ≤ 1.4% Method B: bias +3.9% to +8.4%; CV ≤ 7.6% 30 incurred samples; 29 of 30 within ±20% difference, range -25.8% to +2.9%,		Table X in Report Y Table X in Report Y
List other parameters	NA		

Topics for which clarification is needed

General topics:

1. The suggested tables are to be included in the CTD section 2.7.1. in docx format, as well as an Appendix in the Summary of Biopharmaceutics located in eCTD 2.7.1.
   We do not understand why the tables need to be included twice.
2. Hyperlinks: is it sufficient to include hyperlinks to the specific reports (assay validation, bioanalytical study) or should hyperlinks to the specific report sections be also included in the summary tables?
3. It is not clear whether the tables should only be completed for the drug(s) which are the object of the NDA/BLA or also for other analytes (e.g. DDI probe substrates, for which limited information on the assay lifecycle could be available to the sponsor).

Topics for which clarification is needed (cont.)

Table 1:

1. Should “stock reference” be read as “batch number of the reference standard” or should additional information on stock solutions be included, too?

Table 2a:

1. in section 2.0 of the Guidance it is clearly stated that info should be provided “using one method per analyte per table”. It is not clear if this is also valid in case of multiple analytes determined using the same method. In this case we would recommend to have only one table per method.
2. “Material used for standard calibration curve/QCs and concentration”; is “material” intended as the matrix (e.g. EDTA plasma) or should any additional information, e.g. lot of the reference standard be also provided?
3. “Source and lot of reagents”: we assumed this is limited to the reagents employed in ligand binding assays, only. Is this a correct assumption?

Topics for which clarification is needed (cont.)

Table 2a (cont.):

4. “Cumulative accuracy in 5 QCs”; It is not clear what number “5" refers to. Also, it is not clear what “cumulative” means (e.g. inter-assay?) throughout the document.
5. It is not clear what the meaning of “standard calibration curve performance during A&P runs” in the method performance summary is. In the instructions, it is requested to “provide the number of standard calibrators”.
6. Regarding the stability assessment, it is not clear what level of detail is required: our recommendation is to list only the last accepted stability timepoint, including the related storage temperature.

Table 2b:

1. The purpose of this table is unclear. In particular, what is meant with “performance of calibrators and QCs during accuracy and precision runs” as these are not performed as part of cross validations?
2. Also, our suggestion is not to populate some of the fields, as most of the information is already provided in Table 1.

Proposed guide

It provides instructions on the way the different fields should be populated

Based on our interpretation of the technical requirements defined in the guideline

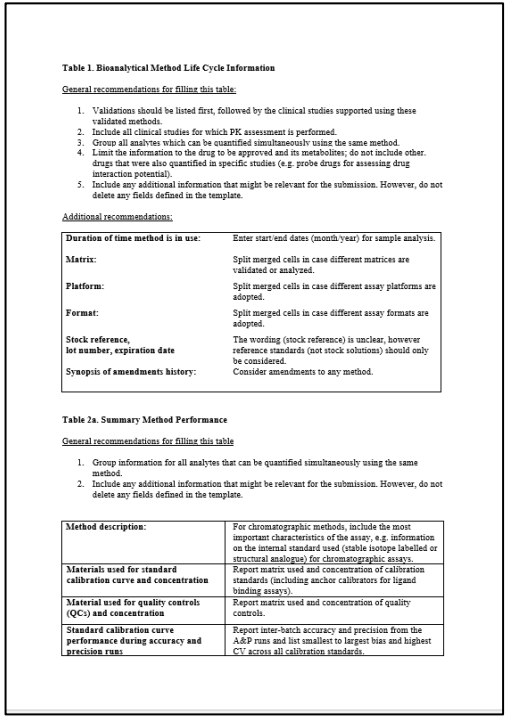


Table 1. Bioanalytical Method Life Cycle Information

General recommendations for filling this table:

1. Validations should be listed first, followed by the clinical studies supported using these validated methods.
2. Include all clinical studies for which PK assessment is performed.
3. Group all analytes which can be quantified simultaneously usinz the same method.
4. Limit the information to the drug to be approved and its metabolites; do not include other. drugs that were also quantified in specific studies (e.g. probe drugs for assessing drug interaction potential).
5. Include any additional information that might be relevant for the submission. However, do not delete any fields defined in the template.

Additional recommendations:

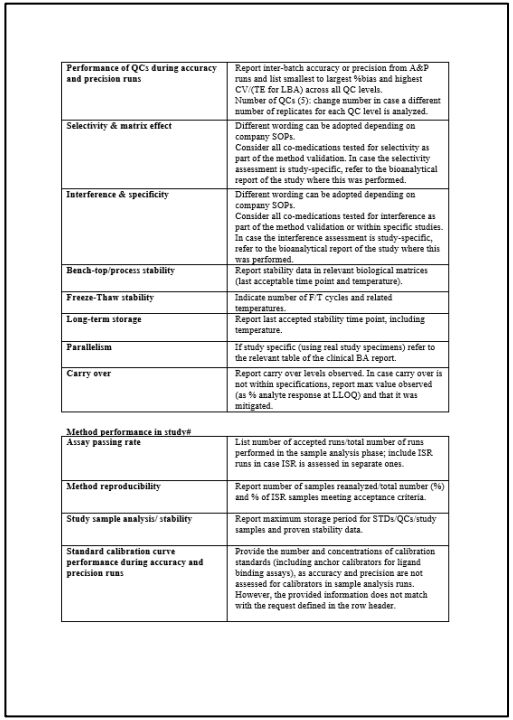
Duration of time method is in use:	Enter start/end dates (month/year) for sample analysis.
Matrix:	Split merged cells in case different matrices are validated or analyzed.
Platform:	Split merged cells in case different assay platforms are adopted.
Format:	Split merged cells in case different assay formats are adopted.
Stock reference, lot number, expiration date	The wording (stock reference) is unclear, however reference standards (not stock solutions) should only be considered.
Synopsis of amendments history:	Consider amendments to any method.

Table 2a. Summary Method Performance

General recommendations for filling this table

1. Group information for all analytes that can be quantified simultaneously using the same method.
2. Include any additional information that might be relevant for the submission. However, do not delete any fields defined in the template.

Method description:	For chromatographic methods, include the most important characteristics of the assay, e.g. information on the internal standard used (stable isotope labelled or structural analogue) for chromatographic assays.
Materials used for standard calibration curve and concentration	Report matrix used and concentration of calibration standards (including anchor calibrators for ligand binding assays).
Material used for quality controls (QCs) and concentration	Report matrix used and concentration of quality controls.
Standard calibration curve performance during accuracy and precision runs	Report inter-batch accuracy and precision from the A&P runs and list smallest to largest bias and highest CV across all calibration standards.



Performance of QCs during accuracy and precision runs	Report inter-batch accuracy or precision from A&P runs and list smallest to largest %bias and highest CV/(TE for LBA) across all QC levels. Number of QCs (5): change number in case a different number of replicates for each QC level is analyzed.
Selectivity & matrix effect	Different wording can be adopted depending on company SOPs. Consider all co-medications tested for selectivity as part of the method validation. In case the selectivity assessment is study-specific, refer to the bioanalytical report of the study where this was performed.
Interference & specificity	Different wording can be adopted depending on company SOPs. Consider all co-medications tested for interference as part of the method validation or within specific studies. In case the interference assessment is study-specific, refer to the bioanalytical report of the study where this was performed.
Bench-top/process stability	Report stability data in relevant biological matrices (last acceptable time point and temperature).
Freeze-Thaw stability	Indicate number of F/T cycles and related temperatures.
Long-term storage	Report last accepted stability time point, including temperature.
Parallelism	If study specific (using real study specimens) refer to the relevant table of the clinical BA report.
Carry over	Report carry over levels observed. In case carry over is not within specifications, report max value observed (as % analyte response at LLOQ) and that it was mitigated.

Method performance in study#

Assay passing rate	List number of accepted runs/total number of runs performed in the sample analysis phase; include ISR runs in case ISR is assessed in separate ones.
Method reproducibility	Report number of samples reanalyzed/total number (%) and % of ISR samples meeting acceptance criteria.
Study sample analysis/ stability	Report maximum storage period for STDs/QCs/study samples and proven stability data.
Standard calibration curve performance during accuracy and precision runs	Provide the number and concentrations of calibration standards (including anchor calibrators for ligand binding assays), as accuracy and precision are not assessed for calibrators in sample analysis runs. However, the provided information does not match with the request defined in the row header.

An example of the information provided in the guide

Table 2a: Summary of method performance (in study)

Standard calibration curve performance during accuracy and precision runs	Provide the number of standard calibrators from LLOQ to ULOQ.

No P/A runs are performed within clinical studies

Standard calibration curve performance during accuracy and precision runs	Provide the number and concentrations of calibration standards (including anchor calibrators for ligand binding assays), as accuracy and precision are not assessed for calibrators in sample analysis runs. However, the provided information does not match with the request defined in the row header.

Where we are now

Communication to the FDA:

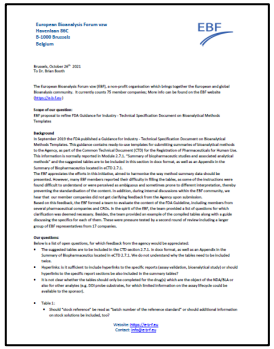
- An official letter was sent to the agency (att. Brian Booth) from the EBF account on 27 Oct 2021. It contained the list of topics for which clarification was deemed necessary.
- The example tables and the guide were also included as *addenda.*

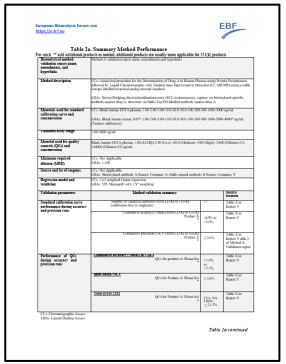
Communication with AAPS:

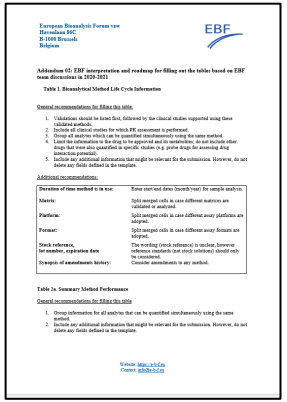
- Faye Vazvaei (on behalf of the AAPS) will present on this topic at the OS in December.

Posting on the EBF website:

- The example tables and the guide will be posted on the EBF homepage (www.e-b-f.eu).







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