Harmonization Team S3
Chromatographic Run Quality Assessment

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Global Bioanalysis Consortium
On harmonization of bioanalytical guidance

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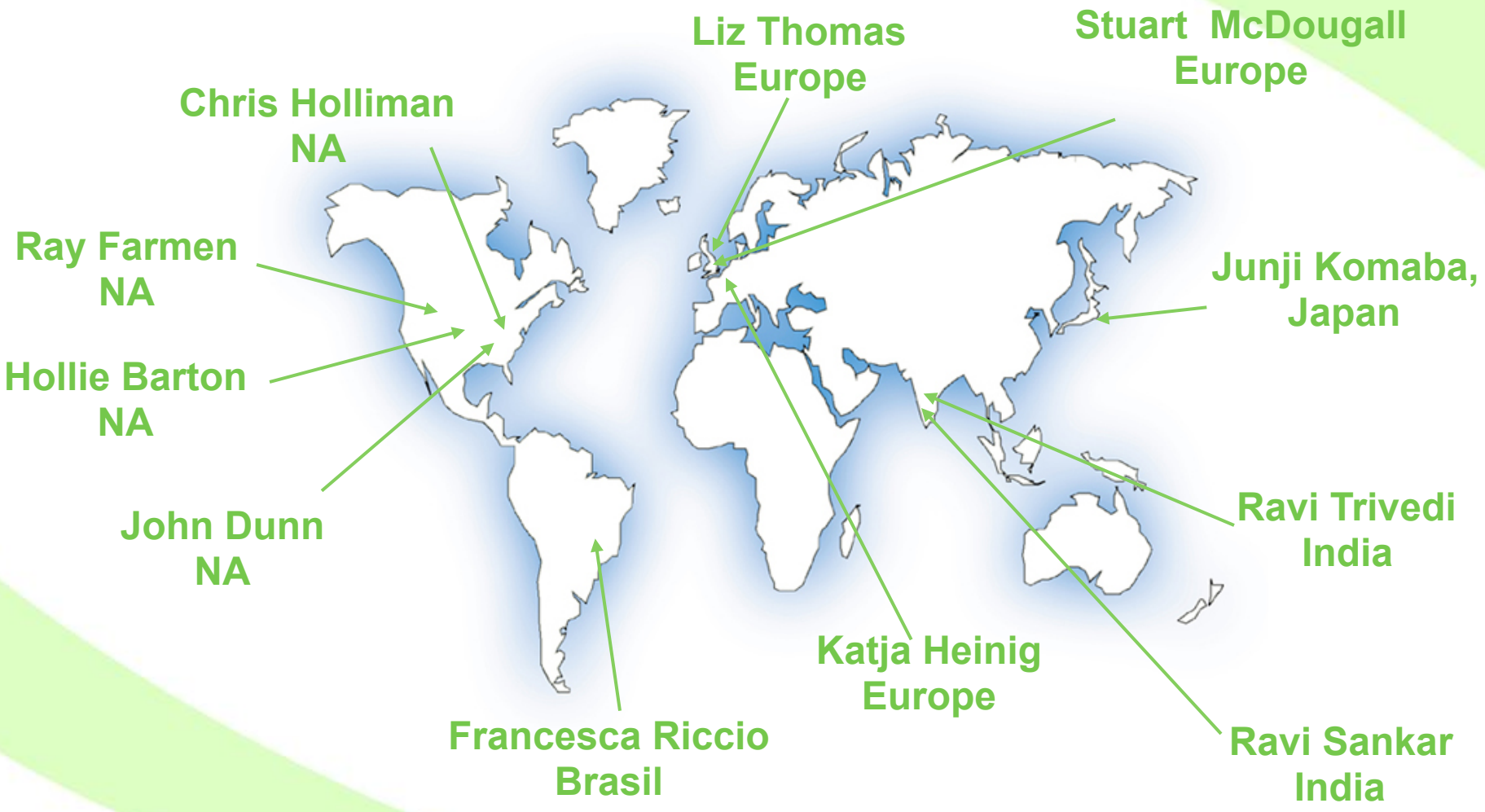

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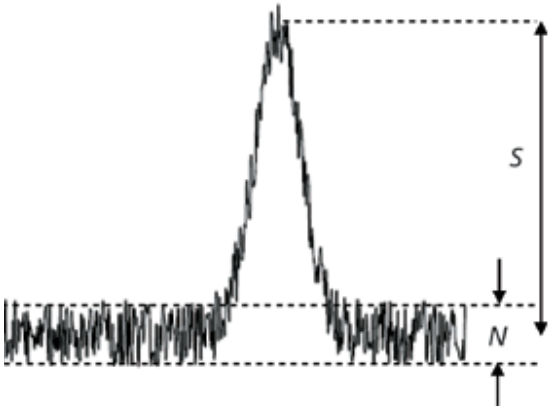
Scope

- Chromatographic assays
  - Can be applied to a wide variety of molecules, including but not limited to, pharmaceuticals, peptides, proteins and biomarkers.
  - The scope encompassed anything that generates a quantitative bioanalytical chromatographic response.
- Chromatographic Separation (GC, LC, SFC, CE, etc)
  - Focused on LC (predominant technique used in bioanalysis). General principles should be translatable to other chromatographic methods
- Chromatographic Detection (UV, FL, EC, MS, etc
  - Focused on MS detection (predominant technique used in bioanalysis). General principles should be translatable to other detection methods.
- Peak Height / Area
  - Focused on Area but did not exclude Peak Height.

Signal to Noise (S/N)

- S/N is related to electronic (instrument) and chemical noise.



- This is different from a specific chemical interference at LLOQ (discrete peak at RT of analyte)
- **S/N used as a 'tool' for bioanalytical method development to ensure robust assay. Definitive evidence of assay performance (fit for use) is precision and accuracy at LLOQ (within ± 20%).**
- Agreed that S/N ~ ≥ 5 for analyte should be target, but in some cases S/N of lower than this may still be acceptable and yield an accurate and precise assay

[1] J Dolan. Enhancing Signal to Noise. LCGC (2010)

Definition of Chromatographic Selectivity

- All relevant regulatory documents refer to selectivity, specificity or both. For consistency with IUPAC [1], Selectivity is correct definition;

'Selectivity is the ability of the bioanalytical method to measure and differentiate the analyte(s) of interest and internal standard in the presence of components which may be expected to be present in the sample' [2]

[1] J Vessman. Pure Appl. Chem. 73, 1381-1386 (2001)
[2] EMEA/CHMP/EWP/192217/2009

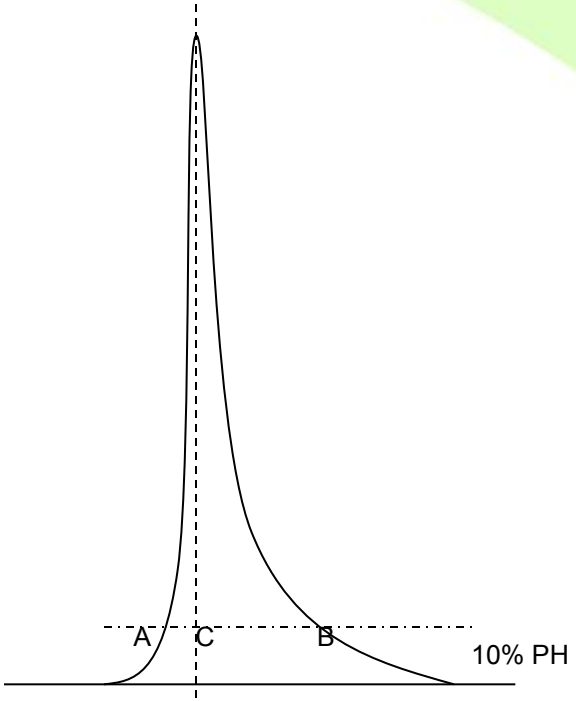
Chromatography

- For chromatography-based assay the following should be considered based on **good scientific practice**;
  - Adequate chromatographic retention, such that a capacity factor of ~5 is obtained (Isocratic ; $2 < K < 10$, Gradient ; $2 < K^* < 10$) [1]
    - Maintaining high capacity factor ensures analyte of interest is adequately separated from interferences (both seen and unseen)
    - Reduces potential impact of ion suppression
  - Adequate chromatographic separation, such that resolution (Rs) of two peaks is at least 2.0 for a robust assay [1]
    - Allows for some chromatographic deterioration in performance.
    - Baseline resolution overcomes potential for integration errors [2,3]
  - Adequate chromatographic efficiency (N), such that the theoretical plate number should be >2000 [1]

[1] Reviewer Guidance; Validation of Chromatographic Methods, FDA CDER 1994
[2] Integration errors in chromatographic analysis, Part I : Peaks of approximately equal size. M. Bicking, LCGC 24, 4, 402-414 (2006)
[3] Integration errors in chromatographic analysis, Part II: Large Peak Size Rations. M. Bicking, LCGC 24, 6, 604-616 (2006)

Chromatography

- Peak asymmetry and tailing factor
  - $TF = AB/2AC_{5\%}$
  - $A_S = BC/CA_{10\%}$
- Asymmetry of an analyte peak is acceptable, provided that the degree of asymmetry observed in calibration and QC samples is reflected in the incurred samples.
- Beyond 2.0, peak symmetry is unacceptable [1, 2].



[1] Reviewer Guidance; Validation of Chromatographic Methods, FDA CDER 1994
[2] J Dolan. The Perfect Method, part 1. LC-GC, 2007

Chromatography

- Consensus agreement regarding the chromatographic terms presented (K, Rs, As, N).
- These parameters are ‘good practice’ to achieve selectivity, rather than formal criteria.
- Peak shape of the calibration and QC samples should be representative of the peak shape of the study samples.
- Important to reiterate that subsequent good peak integration is a result of good chromatography

‘Integration is of use in controlled circumstances, when chromatography is good. They cannot improve bad chromatography: only the analyst can do that’ [1]

[1] N Dyson, Chromatographic Integration Methods, RSC Monographs 1990)

Chromatographic Selectivity

- Any interfering peak at RT of analyte should be ≤ 20% of peak at LLOQ (<5% interference for IS)
  - Verify selectivity using 6 batches of blank matrix (all species) [1,2]
  - Individual subjects (x6) for clinical & pools (x6) for preclinical
- Chromatographic selectivity of assay must be continuously monitored, especially in study samples;
  - Interference from metabolites
  - Interference from co-medications or other exogenous xenobiotics
  - Interference from dose formulation

[1] EMEA/CHMP/EWP/192217/2009
[2] Guidance for Industry 'bioanalytical method validation'. US FDA (CDER/CVM) May 2001

Data Sampling Frequency

- The data sampling frequency can have a significant affect on the accuracy and precision of the assay [1,2,3,4]
  - If the sampling frequency is too slow, peak detail will be lost and measurement accuracy reduced.
  - If the sampling frequency is too high, unwanted baseline noise peaks will be detected and measured.
- A peak is defined by at least 10 points, optimally 15 to 20 [3]
- **Data Sampling Frequency defined in validation must be applied to production (study samples).**
- **Changes in Data Sampling Frequency would constitute a change to the method and would require validation.**

[1] Dyson N, Chromatographic Integration Methods, RSC Monographs 1990)
[2] Meyer, V.R., Advances in Chromatography. P.383. Marcel Deckker, New York, 1995.
[3] Hill, H et al., Chromatographia, 55 Suppl., S79-S81, 2002.
[4] Introduction to modern chromatography 3rd Edition, Snyder, Kirkland and Dolan (2009) Wiley

Data Filtering & Smoothing

- Instrumental measurement consists in two components
  - The signal that contains information about the analyte
  - The noise signal.
- Two types of noise in instrumental analysis;
  - long-term noise; derived from external source variables (temperature, humidity variations, bleed of stationary phase bleed, late eluting compound from prior injections and baseline drift, etc)
  - short term (high frequency) noise; derived from each component of analytical instrument (detector noise, pulsations of the LC system, transducer in/out power source, etc).
- Vendor specific hardware (filtering) and software (smoothing) methods are used to reduce noise and improve the S/N ratio in instrumental analyses.

Data Filtering

- Chromatographic data systems (CDS) use proprietary electronic noise filtering to improve signal to noise and enhance the signal.
- This is effective at the point of data collection and can be considered the raw data.
- **Data Filtering parameters defined in validation must be applied to production (study samples).**
- **Changes in these parameters would constitute a significant change to the method and require validation.**

Data Smoothing

- Smoothing improves the signal and reduces the noise using **post acquisition algorithms** that connect the discrete data points in a well defined manner (e.g. media boxcar, moving average and Savitzky Golay).
  - The most appropriate value for smooth depends on the number of acquisition points by extending the chromatographic peak. The maximum value of window width is five scans.
  - It is necessary to adjust the acquisition parameters to obtain an appropriate number of acquisitions points in peak extension.
  - The number of extension points in the peak will depend on the width of the peak and the data sampling frequency
- The minimum amount of smoothing should be applied to the data.
- **Smoothing iterations and smoothing width defined in validation must be applied to production (study samples).**
- **Changes in these parameters would constitute a significant change to the method and require validation**

Integration

"It should be clear now that integrators are like any other tool – an excellent thing in the **right hands**. What they do best is measure peaks which are suitable for measuring, rapidly and without tedium.

If these measurements are worth making then all subsequent calculations are worth noting and perhaps acting upon.

As long as integrators use perpendiculars and tangents and draw straight baselines beneath peaks, they are of use only in controlled circumstances, when chromatography is good. Even then, the use of integrators requires **vigilance from the operator and skill in assessing and assigning parameters**. Integrators cannot improve bad chromatography: only the analyst can do that – and at the end of the day that's what they are paid for" [1]

[1] N Dyson, Chromatographic Integration Methods, RSC Monographs 1990

Integration

- To ensure correct peak integration it accepted that an analyst may need to adjust certain integration parameters;
  - Changes in instrument response (ageing, sensitivity, etc)
  - Changes in chromatographic performance (minor change in peak shape, change in noise, etc)
  - Manufacturing differences between instruments of same vendor & platform (e.g. API4000 vs. API4000).
  - It is not appropriate to set guidance on use of specific integration parameters (Noise Threshold, etc) as these are method specific.
- Integration parameters used in assay validation should be considered as a starting position for run integration. **It is scientifically invalid to assume that chromatographic integration parameters must be fixed.**
- Integration of chromatographic peaks should be consistent for calibration, QC and incurred samples within a run.

Initial Integration

- Definition; Process by which the area (or height) of a chromatographic peak is adequately defined by a trained bioanalyst using the most appropriate integration parameters prior to regression.
- The definition includes;
  - Automated integration (integration parameters affecting all chromatograms are adjusted on a run by run basis)
  - Modified automated integration (integration parameters are adjusted on a chromatogram by chromatogram basis)
  - Manual (drag and drop) integration on a chromatogram by chromatogram basis.
- Automatic, modified and manual chromatograms should be retained.
- The method and reason for adjusting integration parameters should be documented
- Integration process should be defined in SOP

Reintegration

- Definition; The process that occurs post regression (i.e. the back-calculated concentration, subsequent acceptance of calibration and QC samples, and hence acceptance or rejection of the run, is defined) following rejection of the initial integration.
  - Reintegration should only occur after documented peer review by trained personnel.
  - The reintegration as well as the initial integration (including any modified integration) must be retained
  - Reintegration process should be defined in SOP
- Process control (such as access rights and audit trail) will be required to ensure validity.

Integration, Reintegration and Regression

- There is a perception within the industry that the integration process should be fully defined (i.e. ‘fixed’ parameters). However, this has potential to have a negative effect on the overall quality of the peak integration, especially when there are many other parameters affecting the process such as peak shape, response, sensitivity, etc.
- We recognize the potential for misconduct (peak skimming & peak enhancing), but need to clearly distinguish and educate others on the benefits of separating chromatographic integration from regression (and reintegration)
- Chromatographic integration parameters are important, and provided that they are consistently applied and documented, the ultimate control of the assay on a run by run basis is made by the calibration, QC and internal standard acceptance criteria.

Internal Standard Response

- GBC-S3 recommend that the wording of EMEA guidance is sufficient at this moment in time [1]
  - Reasons for study sample reanalysis: internal standard response significantly different from the response for the calibration standard and QC samples, if such criteria have been pre-defined in a SOP.
- An SOP describing Internal Standard Response should be in place, but this does not necessarily need to have restrictive mathematical criteria (i.e. mean +/- 50%, or similar). If SOP in place, then applicable to both study sample analysis and validation
- Scientific judgment should take precedence and should be able to discriminate between missing Internal Standard and double spike (as common events).
- The impact of Internal standard drift within a run, internal standard response changes in specific subject(s) or other Internal Standard Response events cannot be mandated by a simple mathematical rule.

[1] EMEA/CHMP/EWP/192217/2009

Audit Trail

"Paper or 'electronic' trail that gives a step by step documented history of a transaction. It enables an examiner to trace the data source document. The presence of a reliable and easy to follow audit trail is an indicator of good internal controls instituted by a firm, and forms the basis of objectivity [1]"

- Regulations regarding audit trail relate to predicate rules and are applicable to chromatographic run quality [2].
- Audit trails are not routinely examined, unless in time of inspection.
- Can be viewed as automated quality process 'oversight'.
- Recommend to have predefined reasons for improved clarity of audit trail (either by SOP or specific training)

[1] WHO Library Cataloguing-in-Publication Data Handbook: good laboratory practice (GLP): quality practices for regulated non-clinical research and development -2nd ed. 1.Laboratories - organization and administration. 2.Laboratories - handbooks. 3.Laboratories techniques and procedures. 4.Manuals. I.UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. ISBN 978 92 4 154755 0 (NLM classification: QY 25)
[2] CT Vishwanathan. Regulatory observations in bioanalytical determinations. Bioanalysis (2010) 2(7), 1325-1329

Chromatographic Data Review

- Peer review of a batch (post regression) should include 100% audit of chromatograms for consistency of both chromatography and integration.
- Bioanalyst performing integration must have documented evidence of training in both general principals of chromatographic integration and specifics of system being used (Waters QuanLynx, Sciex Analyst, Thermo LCQuan, etc).
- Peer reviewer must have documented evidence and experience of chromatographic data audit
- All chromatograms (both initial and final), together with associated integration parameters, should be available for regulatory review if requested [1,2]

[1] EMEA/CHMP/EWP/192217/2009
2] CT Vishwanathan et al. Quantitative Bioanalytical Methods Validation and Implementation; Best Practices for Chromatographic and Ligand Binding Assays. AAPS Journal 2007, 9(1), E30-E42

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- Dyson, N. Chromatographic Integration Methods. RSC Chromatography Monographs. 1990. ISBN 0-85186-587-9
- Introduction to Modern Chromatography. 3rd Edition, Snyder, Kirkland and Dolan (2009) Wiley

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- Integration errors in chromatographic analysis, Part II: Large Peak Size Rations. M. Bicking, LCGC 24, 6, 604-616 (2006)
- CT Vishwanathan et al. Quantitative Bioanalytical Methods Validation and Implementation; Best Practices for Chromatographic and Ligand Binding Assays. AAPS Journal 2007, 9(1), E30-E42

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- ANVISA CP33 (draft for new resolution), 2011
- J Dolan. The Perfect Method, part 1. LC-GC, 2007
- WHO Library Cataloguing-in-Publication Data Handbook: good laboratory practice (GLP): quality practices for regulated non-clinical research and development. 2nd ed. 1.Laboratories - organization and administration. 2.Laboratories - handbooks. 3.Laboratories techniques and procedures. 4.Manuals. I.UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. ISBN 978 92 4 154755 0 (NLM classification: QY 25)
- CT Vishwanathan. Regulatory observations in bioanalytical determinations. Bioanalysis (2010) 2(7), 1325-1329
- Fabio Garofolo et al. 2011 White paper on recent issues in bioanalysis and regulatory findings from audits and inspections. Bioanalysis (2011) 3(18) 2081-2096 .

Citations

- Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009)- effective Feb 2012
  - Chromatogram integration and re-integration should be described in a SOP. Any deviation should be discussed in the analytical report. Chromatogram integration parameters and in case of re-integration, initial and final integration data should be documented at the laboratory and should be available upon request. For further guidance reference is made to the "Reflection Paper for Laboratories That Perform The Analysis Or Evaluation of Clinical Trial Samples" EMA/INS/GCP/532137/2010.

Citations

- Guidance for Industry ‘bioanalytical method validation’. US FDA (CDER/CVM) May 2001
  - Sample data reintegration: An SOP or guideline for sample data reintegration should be established. This SOP or guideline should explain the reasons for reintegration and how the reintegration is to be performed. The rational for the reintegration should be clearly described and documented. Original and reintegration data should be reported.
  - Documentation for reintegrated data. Documentation should include the initial and repeat integration results, the method used for reintegration, the reported result, assay run identification, the reason for the reintegration, the requestor of the reintegration, and the manager authorizing reintegration. Reintegration of a clinical or preclinical sample should be performed only under a predefined SOP

Citations

- Crystal City III; Quantitative Bioanalytical Methods. CT Viswanathan et al.
  - Documentation at the Analytical Site Should Include; Reintegrated chromatograms should be explicitly identified. The reason for reintegration and the mode of reintegration should be documented. The original and reintegrated chromatograms should be retained ideally as electronic records.
  - Regarding chromatographic methods, source documentation should include original and reintegrated chromatograms for accepted runs, along with the reason for changing integration parameters across a run or for individual samples within a run. Disabling electronic audit trails that record changes to integration parameters is not acceptable

Citations

- 2011 White paper on recent issues in bioanalysis and regulatory findings from audits and inspections. Bioanalysis (2011) 3(18) 2081-2096 . Fabio Garofolo et al
  - Manual integration has been discussed numerous times in the past, including the 2009 white paper on bioanalysis, but the topic was discussed again briefly and an update since 2009 was needed. Generally speaking, it is accepted by the industry and regulatory authorities that integration done automatically is preferred, but that it may sometimes be appropriate to perform manual integration. It was agreed that manual integration is generally acceptable as long as: it is done before the results are regressed; is justified and is documented in a controlled fashion; and uses good scientific judgment and common sense.