World Health Organization

GOOD CHROMOTOGRAPHY PRACTICES

(February 2019)

DRAFT FOR COMMENTS

Please send any comments you may have to Dr S. Kopp, Group Lead, Medicines Quality Assurance, Technologies Standards and Norms (kopps@who.int), with a copy to Ms Sinéad Jones (jonessi@who.int) **by 30 March 2019**.

Medicines Quality Assurance working documents will be sent out electronically only. They will also be placed on the Medicines website for comment under "Current projects". If you have not already received our draft working documents, please send your email address (to jonessi@who.int) and we will add you to our electronic mailing list.

SCHEDULE FOR DRAFT WORKING DOCUMENT QAS/19.791:

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Description of Activity	**Date**
Following a recommendation by the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP) by Dr André Van Zyl, a member of the ECSPP, the WHO Secretariat was recommended to develop a new guidance on good chromatography practices.	October 2018
Preparation of first draft working document.	December 2018 - February 2019
Mailing of working document to the Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations (EAP) inviting comments and posting of the working document on the WHO website for public consultation.	February – May 2019
Consolidation of comments received and review of feedbacks. Preparation of working document for discussion.	June 2019
Discussion of working document and feedbacks received during the informal Consultation on Good Practices for Health Products Manufacture and Inspection.	July 2019
Revision of the working document based on comments received during the informal Consultation on Good Practices for Health Products Manufacture and Inspection.	End of July 2019
Mailing of revised working document to the EAP inviting comments and posting the working document on the WHO website for public consultation.	August – September 2019
Consolidation of comments received and review of feedbacks. Preparation of working document for discussion.	End of September 2019
Presentation to the Fifty-fourth meeting of the ECSPP.	14 -18 October 2019
Any other follow-up action as required.	

GOOD CHROMOTOGRAPHY PRACTICES

1. INTRODUCTION AND SCOPE

The use of chromatography methods (such as High Pressure Liquid Chromatography (HPLC) and Gas Chromatography (GC)) in quality control laboratory analysis has increased significantly in recent years.

HPLC and GC methods are used in, for example, the identification of materials and products, for determination of assay and related substances in materials and products, as well as in validation such as process validation and cleaning validation.

Due to the criticality of the results obtained through chromatography, manufacturers should ensure that the data acquired is accurate and reliable. Results should meet ALCOA+ principles (i.e. . attributable, legible, contemporaneous, original and accurate).

This guideline provides information on good practices to be considered in the analysis of samples when chromatographic systems are used. The principles should be applied in the analysis of, for example, raw materials, starting materials, intermediates, in-process materials and finished products.

The principles contained in this guideline are applicable to all types of chromatographic analysis used in, for example, assay determination, testing for related substances and impurities, process validation, cleaning validation, cleaning verification and stability testing.

2. GLOSSARY

ALCOA

A commonly used acronym for “attributable, legible, contemporaneous, original and accurate”.

ALCOA+

A commonly used acronym for "attributable, legible, contemporaneous, original and accurate" which puts additional emphasis on the attributes of being complete, consistent, enduring and available – implicit basic ALCOA principles.

Backup

A backup means a copy of one or more electronic files created as an alternative in case the original data or system are lost or become unusable (for example, in the event of a system crash or corruption of a disk). It is important to note that backup differs from archival in that backup copies of electronic records are typically only temporarily stored for the purposes of disaster recovery and may be periodically overwritten. Such temporary backup copies should not be relied upon as an archival mechanism.

Calibration

The set of operations that establish, under specified conditions, the relationship between values indicated by an instrument or system for measuring (especially weighing), recording and controlling, or the values represented by a material measure, and the corresponding known values of a reference standard. Limits for acceptance of the results of measuring should be established.

Chromatographic column

A tube commonly filled with a stationary phase over which a sample and mobile phase move, used in chromatographic analysis.

Data

All original records and true copies of original records, including source data and metadata and all subsequent transformations and reports of these data, which are generated or recorded at the time of the GMP activity and allow full and complete reconstruction and evaluation of the GMP activity. Data should be accurately recorded by permanent means at the time of the activity. Data may be contained in paper records (such as worksheets and logbooks), electronic records and audit trails, photographs, microfilm or microfiche, audio- or video-files, or any other media whereby information related to GMP activities is recorded.

Exponential skim

The creation of a curvature in the skim line in an attempt to approximate the underlying baseline of the parent peak.

Exponential curve fitting

The drawing of a curve using an exponential equation through the start and end of the child peak. The curve passes under each child peak that follows the parent peak; the area under the skim curve is subtracted from the child peaks and added to the parent peak.

Front peak skim

The process of integrating child peaks on the front (upslope) of a peak subject to specified criteria.

Integration

The process of applying specified parameters for chromatographic peaks (determination of height, area and retention time).

Metadata

Metadata is data about data that provides the contextual information required to understand those data. These include structural and descriptive metadata. Such data describe the structure, data elements, interrelationships and other characteristics of data. They also permit data to be attributable to an individual. Metadata necessary to evaluate the meaning of data should be securely linked to the data and subject to adequate review. For example, in weighing, the number 8 is meaningless without metadata, such as, the unit, milligram, etc. Other examples of metadata include the time/date stamp of an activity, the operator identification (ID) of the person who performed an activity, the instrument ID used, processing parameters, sequence files, audit trails and other data required to understand data and reconstruct activities.

Peak valley ratio

The peak to valley ratio is a measure of quality indicating how well the peak is separated from other substance peaks.

Qualification

Documented evidence that premises, systems or equipment are able to achieve the predetermined specifications properly installed, and/or work correctly, and lead to the expected results.

Rear peak skim

The process of integrating child peaks on the tail (downslope) of a peak subject to specified criteria.

Restoration

The process of retrieving electronic data that had previously been backed-up and presented in a readable format.

Source data

Original data obtained as the first-capture of information, whether recorded on paper or electronically.

Straight line skim

The process of drawing a straight line through the start and end of a child peak. The height of the start of the child peak is corrected for the parent peak slope. The area under the straight line is subtracted from the child peak and added to the parent peak.

Tangential skim

The process of integrating a small peak located on the tailing edge of a larger peak. The baseline of the small peak becomes a tangent drawn from the valley of the larger peak to the tangent point on the chromatogram.

Validation

Action of proving and documenting that any process, procedure or method actually and consistently leads to the expected results.

Valley height ratio

Valley height ratio is the ratio of the height of the child peak above the baseline to the height of the valley above the baseline. This ratio must be smaller than the specified value for the child peak to be skimmed.

3. CHROMATOGRAPHIC SYSTEMS

3.1. Manufacturers should select, install and use chromatographic systems that are appropriate for the intended use.

3.2. Vendor qualification should ensure that hardware and software are suitable for their intended application and that after-sales services will be available.

3.3. Valid agreements should specify the respective responsibilities between the purchaser and supplier.

3.4. Systems should meet regulatory requirements and expectations for Good Manufacturing Practices, including but not limited to, ensuring that data are acquired, processed and stored in accordance with national legislation and ALCOA+ principles.

4. QUALIFICATION AND VALIDATION

4.1. The scope and the extent of validation and qualification of chromatographic systems should be determined based on risk management principles.

4.2. The approach to, and execution of validation and qualification, should be described in an authorized document such as a validation master plan.

4.3. All stages of qualification should be considered and may include, for example, user requirement specifications (URS), design qualification (DQ), factory acceptance test (FAT), site acceptance test (SAT), installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ). (*See also the Guideline for Computerized Systems*).

4.4. Validation and qualification should be described in protocols and recorded in reports. Reports should contain documented evidence such as screen shots, printouts or other source data of tests executed as part of validation and qualification.

4.5. The data should provide evidence of the consistency of performance of the system, and reliable and accurate results.

4.6. Parameters such as, but not limited to, password control, audit trail, access and privileges, should be described and verified during validation and qualification.

4.7. Chromatographic systems should be calibrated at periodic intervals in accordance with written procedures. Records should be maintained.

4.8. Root cause analysis, impact and risk assessment should be done when any calibration parameter is found out of calibration or not meeting the predefined limits. Appropriate corrective and preventive action (CAPA) should be taken.

5. ACCESS AND PRIVILEGES

5.1. There should be a standard operating procedure (SOP) for the creation and deletion of user groups and users of the chromatographic system, indicating the relevant privileges allocated to each user. Records should be maintained.

5.2. An up-to-date matrix of user groups and users should be maintained.

5.3. Users in each group should be appropriately qualified for the responsibility and privileges given.

5.4. Manual records of user groups, users and their privileges should be concordant with electronic data.

5.5. Where required, justification should be provided for privileges granted to user groups or users.

6. AUDIT TRAIL

6.1. Chromatographic systems should have (an) audit trail(s).

6.2. Full audit trails should be enabled from the time of installation of software.

6.3. Audit trails should remain enabled throughout the life cycle of a chromatographic system.

6.4. Audit trails should be reviewed in accordance with an SOP. There should be evidence of the regular (each chromatographic analysis) and periodic review (at random checks at specified intervals) of audit trails.

6.5. Audit trails are part of metadata and should be stored as part of the data set for all chromatographic analysis.

7. DATE AND TIME FUNCTIONS

7.1. Chromatographic systems should have date and time functions enabled.

7.2. The date and time function should be locked and access to change the date and time should be controlled. This includes time zones.

7.3. All actions on chromatographic systems should be date and time-tracked.

8. ELECTRONIC SYSTEMS

Note: This includes computerized systems

8.1. Electronic systems used in chromatographic systems should be suitable for their intended use.

8.2. Where possible, all chromatographic systems should be linked to a network.

8.3. Stand-alone systems should be appropriately managed. Risk assessment should be done to ensure that sufficient controls are in place to eliminate the risks associated with stand-alone systems. These include, but are not limited to, access, privileges, date and time function, audit trail, back-up and data management

8.4. Electronic Data Management Systems (EDMS) should be considered for the appropriate management of data, including acquisition, processing and storage of data. EDMS should be appropriate for their intended use and ensure the accuracy and reliability of data obtained and processed.

8.5. Appropriate procedures should be followed when a new electronic system is taken into use. Procedures should also be followed for the removal of a system from use. Records should be maintained.

9. SOLVENTS, BUFFER SOLUTIONS AND MOBILE PHASES

9.1. Solvents, buffer solutions and mobile phases should be prepared, stored and used in accordance with authorized specifications, procedures and pharmacopoeia. These should be used within their validated shelf life.

9.2. Records for their preparation should be maintained.

9.3. Chemicals, reagents and other materials used should be of appropriate grade and quality.

9.4. Mobile phases should be filtered and degassed when required.

10. COLUMN MANAGEMENT

10.1. Columns used in chromatography should be appropriate for their intended use.

10.2. Columns should be purchased from approved suppliers.

10.3. Columns should be verified on receipt and checked for their suitability prior to use.

10.4. Columns, tubing and fittings should be appropriate to ensure that the system performs as expected.

10.5. Backpressure and flow rates should be appropriate for the column to be used and specified in specifications and test procedures.

10.6. Column efficiency should be appropriate (number of theoretical plates) to ensure good chromatography.

10.7. Risks associated with columns, such as “chiral columns”, should be controlled.

10.8. Flow rate, loading capacity and backpressure should be appropriate to ensure desired and constant retention time.

10.9. The use of columns should be recorded in a traceable manner. This includes, for example, a unique identification number, number of injections and washing.

10.10. Columns should be washed (cleaned or flushed) according to defined procedures which define the steps and parameters, such as sequence and flow rate.

10.11. Columns should be stored in a manner that ensures that they are not damaged.

10.12. Equilibrating columns before, and temperature control of column and mobile phase during analysis, should be done when specified.

10.13. Removal of contaminants and regeneration of columns should only be considered when the appropriate procedures for this had been developed.

11. SAMPLE MANAGEMENT

11.1. Sample management (including the receiving and preparation of samples) should be considered as important aspects in good chromatography practices.

11.2. Samples for analysis, received in the laboratory, should be entered in an appropriate record which ensures the traceability of the sample details and analysis.

11.3. Samples should be stored under appropriate conditions.

11.4. Samples, blanks and standards should be prepared in accordance with the authorized specification or standard test procedure. Records for the preparation should be maintained.

11.5. Official, secondary or working standards used should be traceable to records maintained for their purchase, preparation, storage and use.

11.6. Standard solutions prepared for use in chromatography should be used within their validated shelf life.

11.7. Validated or verified (as applicable) analytical methods should be used.

11.8. The sample set (sample sequence) should be defined and vials with standard solution(s), sample solution and blank solution should be verified to ensure the correct sequence of injections in the chromatographic system.

11.9. Where carry-over or interference in analysis is relevant, suitable precautions should be taken.

11.10. The use of "trial injections", "system check injections" or other injections that are not specified as part of a sample set, is not recommended. In exceptional cases where these are employed, this should be clearly described in an authorized procedure. Only standard solutions may be used for this purpose. The electronic record of source data should be saved and stored together with the source data of the sample set for analysis.

11.11. System suitability (SST) should be part of the sample set. The SST should be performed as described in the respective pharmacopoeia monograph or validated in-house specification and standard test procedure. SST should meet the predetermined acceptance criteria.

11.12. Standard solution injections (bracketing standards) should be consider and included in the sample set, at defined intervals. The frequency of inclusion in a sample set should be justified. Acceptance criteria should be set and monitored.

11.13. Acceptance criteria should be set for SST, bracketing standards, deviation from relative retention, and any other aspect that may be deemed necessary for the chromatographic analysis. This includes acceptability of peak shapes.

11.14. Where blank interferences are detected, these should be within limits.

12. CHROMATOGRAPHIC METHODS (ACQUISITION AND PROCESSING)

12.1. Chromatographic methods selected should be appropriate for their intended use.

12.2. Non-pharmacopoeia methods should be developed, validated and detailed on standard procedures. These should be followed by qualified, experienced personnel.

12.3. Where possible, methods should be created and saved by appointed personnel, in the chromatographic system. Methods selected for analysis should not be modified unless approved for the intended purpose by authorized personnel.

12.4. Results acquired should be processed through validated methods. Where methods for acquisition and processing are different, selected methods should be traceable and reflected in the audit trail.

12.5. Methods should be proven to remain in a validated state throughout the life cycle of the method.

13. CHROMATOGRAPHIC PEAKS

13.1. Chromatographic analysis should meet ALCOA+ principles.

13.2. Factors considered during method validation should include

- recovery experiments;
- slope sensitivity;
- peak width;
- bunching factor;
- area reject;
- noise threshold; and
- area threshold.

13.3. Peaks should be reviewed for acceptability according to policies and procedures, including recommendations and requirements from national regulatory authorities, pharmacopoeia and analytical validation.

13.4. Where more than one column has to be used in complex analysis, the procedure and instructions should be clear in order to ensure that no errors are made during analysis.

13.5. Procedures should include the recommendations and considerations for good chromatography practices as described in this guideline, with specific reference to policies, acceptance limits (as appropriate) and ALCOA+.

14. PEAK INTEGRATION

14.1. Peak areas in analytical chromatograms should be accurately and consistently integrated in a scientifically sound manner.

14.2. Where possible, HPLC and GC instruments should be interfaced with computerised chromatographic data capturing and processing systems which are capable of performing the integration process automatically.

14.3. The same integration parameters should be applied to all peaks in a sample set or sample sequence unless otherwise scientifically justifiable.

14.4. Personnel should select appropriate values for the parameters (such as, slope sensitivity, noise threshold, peak width, area threshold and bunching factor and skim ratio) which are used by the processing software to define the respective chromatographic peaks.

14.5. To facilitate the accurate integration of chromatographic peaks, it is necessary that all of the peaks are fully separated. If quantitative data must be obtained from unseparated peaks, the laboratory should have clear policies as to how such peaks should be integrated. This should include a description as to when it is acceptable to use different functions for integrating unresolved peaks, such as:

- tangential skim;
- exponential skim;
- exponential curve fitting;
- straight line skim;
- front peak skim;
- rear peak skim;
- peak valley ratio; and
- valley height ratio.

14.6. Validated methods, specified chromatographic conditions and good chromatography practices should facilitate obtaining symmetrical peaks. Where fronting, tailing, split peaks or other types of peaks are observed, these should be investigated, the root cause identified and appropriate CAPA taken.

14.7. Where manual integration has to be done, authorized procedures should be followed. Records should be maintained which include the authorization and justification for manual integration.

14.8. Using a procedure to integrate peak height or area by manually setting the baseline using chromatographic software should only be allowed in exceptional cases. Only a selected number of users should be granted privileges to do so. Records and justification should be given when this procedure is followed.

14.9. Where smoothing is applied, the type of "filter" used and extent of smoothing should be justified.

15. CLEANING VALIDATION

Note. For recommendations relating to cleaning validation, see Annex 4, Supplementary Guidelines on Good Manufacturing Practices: Validation (WHO Technical Report Series, No. 937, 2006, Appendix 3).

15.1. Where possible, specific methods should be developed, validated and then used in cleaning validation and cleaning verification.

15.2. Chromatographic methods selected should be specific and appropriate to detect the presence of the substance to be analysed.

15.3. Data and results should be managed in accordance with these guidelines and other relevant guidelines relating to cleaning validation, chromatography and applicable chapters in pharmacopoeia.

15.4. Data and results should be retained for appropriate times to enable inspection thereof.

16. DATA MANAGEMENT

16.1. Chromatographic data acquired should be attributable, legible, original and accurate.

16.2. Procedures should be followed for the processing of data and reporting of results.

16.3. Data should be backed up according to procedures and records maintained as proof thereof. Special care should be taken to ensure frequent back up of data from stand-alone systems to prevent loss of data.

16.4. Data should be safely stored, including control over access to data. Backed-up data should be randomly selected for restoration and verification, at defined intervals.

16.5. Where appropriate, hard copies of data (including metadata) and results should be retained as part of the analytical report reflecting analysis performed.

Note: See other guidelines addressing computerized systems, data integrity and good documentation practices.

16.6. Procedures should be in place to allow for recovery of chromatographic data in case of disasters such as instrument failure, viruses, hardware or software failure and power failure.

16.7. Complete data should be retained for appropriate periods of time to allow for data verification, registration or other reasons.

Acronyms

ALCOA	attributable, legible, contemporaneous, original and accurate
CAPA	corrective and preventive action
DQ	design qualification
EDMS	Electronic Data Management Systems
FAT	factory acceptance test
GC	gas chromatography
HPLC	High Pressure Liquid Chromatography
IQ	installation qualification
OQ	operational qualification
PQ	performance qualification
SAT	site acceptance test
SOP	standard operating procedure
SST	system suitability
URS	user requirement specifications

Further reading

The International Pharmacopoeia, http://apps.who.int/phint/2018/index.html

European Pharmacopoeia, https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition

United States Pharmacopoeia, http://www.usp.org/

Appendix 5: Validation of Computerized Systems Annex 3 (Technical Report Series No. XX, Annex 3, 2019)

Guidance on Good Data and Record Management Practices (WHO Technical Report Series No. 996, 2016, Annex 5)

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