Annex 4

Good chromatography practices

1. Introduction and scope

1.1 The use of chromatography methods such as high-performance liquid chromatography, also referred to as high-pressure liquid chromatography (HPLC), and gas chromatography (GC) in quality control laboratory analysis has increased significantly in recent years. Observations during inspections have shown that there was a need for a specific good practices (GXP) document.

1.2 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. *Note*: Although thin-layer chromatography methods are also used, this approach is not specifically addressed in detail in this document.

1.3 Owing to the criticality of the results obtained through chromatography, it must be ensured that the data acquired meet ALCOA+ principles (i.e. attributable, legible, contemporaneous, original and accurate, with additional emphases [see Glossary]).

1.5 This document provides information on GXP to be considered in the analysis of samples when chromatographic methods and 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.

1.6 The principles contained in this guideline are applicable to general 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

The definitions given below apply to the terms used in this guideline that are not defined in existing WHO terms and definitions databases. They may have different meanings in other contexts. *Note*: For general definitions relating to chromatography, see the relevant pharmacopoeia recognized by the national medicines regulatory authority.

ALCOA. A commonly used acronym for "attributable, legible, contemporaneous, original and accurate".

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

audit trail. A form of metadata that contains information associated with actions that relate to the creation, modification or deletion of GXP records. An audit trail provides for secure recording of life-cycle details such as creation, additions, deletions or alterations of information in a record, either paper or electronic, without obscuring or overwriting the original record. An audit trail facilitates reconstruction of the history of such events relating to the record, regardless of its medium, including the "who, what, when and why" of the action.

back-up. 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 back-up differs from archival, in that back-up copies of electronic records are typically only temporarily stored for the purposes of disaster recovery and may be periodically overwritten. Such temporary back-up 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.

data. All original records and true copies of original records, including source data and metadata and all subsequent transformations and reports of these data, that are generated or recorded at the time of the good manufacturing practices (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.

data integrity. The degree to which data are complete, consistent, accurate, trustworthy and reliable and to which these characteristics of the data are maintained throughout the data life-cycle. The data should be collected and maintained in a secure manner, such that they are attributable, legible, contemporaneously recorded, original or a true copy and accurate. Assuring data

integrity requires appropriate quality and risk management systems, including adherence to sound scientific principles and good documentation practices.

metadata. Data about data that provide the contextual information required to understand those data. Metadata necessary to evaluate the meaning of data should be securely linked to the data and subject to adequate review. 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.

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

sample set. The combination of samples, standards and blanks prepared for analysis, which includes the specified sequence to be injected or analysed.

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

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

3. Chromatographic systems

3.1 Chromatographic systems should meet regulatory and GXP requirements. This should include, for example, ensuring that data are acquired, processed and stored in accordance with ALCOA+ principles (see Glossary).

3.2 Supplier selection and vendor qualification should ensure that hardware and software are suitable for their intended application.

3.3 Valid agreements should specify the respective responsibilities between the purchaser and supplier and include arrangements for after-sales services.

3.4 Chromatographic systems selected, installed and qualified should be appropriate for their intended use.

3.5 The environment in which such systems are placed should be appropriate to support their performance. This may include, for example, control of temperature and relative humidity in the area.

4. Qualification, validation, maintenance and calibration

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

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).

4.4 Validation and qualification should be described in protocols and recorded in reports. Reports should contain documented evidence and include, for example, screenshots, printouts or other source data and metadata 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 Maintenance, preventive maintenance and calibration of chromatographic systems should be done in accordance with written procedures. Records should be maintained.

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

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 record of user groups and users should be maintained.

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

5.4 Where required, justification should be provided for privileges granted to user groups or users, including all exceptions.

5.5 User privileges reflected in written procedures should be a true reflection of the privileges allocated electronically.

5.6 Administrator access rights should not be given to other users on the system.

6. Audit trail

6.1 Chromatographic systems should have an audit trail(s) which reflect(s), for example, users, dates, times, original data and results, changes and reasons for change.

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

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

6.3 Audit trails should be reviewed in accordance with an SOP and include systems and project audit trails. There should be evidence of regular review of an audit trail (for example, each sample sequence or sample set in chromatographic analysis) and of periodic review of audit trails. (Periodic review should be done at specified intervals, based on risk management principles.)

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

7. Date and time functions

7.1 Chromatographic systems should have date and time functions enabled from the time of installation of the software.

7.2 The date and time function should be locked, and access to change the date and time should be controlled. (This includes changes to time zone setting.)

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

8. Electronic systems

Note: This includes computerized systems.

8.1 Written 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.

8.2 Software selected, installed and applied for acquisition, processing and calculation of results should be suitable for its intended use, validated, and render results meeting regulatory, GXP and ALCOA+ principles.

8.3 It is preferable that all chromatographic systems be linked to a network system where data are stored and managed on a centralized server.

8.4 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, data back-up and data management.

8.1. 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 acquired and processed.

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 and procedures and a relevant pharmacopoeia recognized by the national medicines regulatory authority. These should be used within appropriate, scientifically justifiable timelines.

9.2 Records for their preparation and use should be maintained.

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

9.4 Liquid mobile phases should be filtered, degassed and pressurized when required.

9.5 Carrier gases used for gas chromatography should have the appropriate purity and be suitable for their intended use.

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 initial receipt and checked for their suitability as part of the chromatographic system, prior to use in analysis.

10.4 Tubing and fittings should be appropriate to ensure that the system performs as expected.

10.5 The number of theoretical plates (column efficiency) should be monitored to ensure efficiency is obtained for acceptable chromatography.

10.6 Columns should be equilibrated before the analysis. The column oven (and column) temperature should be controlled when specified in the analytical procedure.

10.7 The required flow rate should be specified in relevant test procedures. It should be appropriate for the column to be used, to ensure optimal chromatographic separation without exceeding recommended maximum backpressure.

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

10.9 Columns should be washed (cleaned or flushed) according to defined procedures describing the steps and parameters, such as sequence, temperature, flow rate and time.

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

11. Sample management and sample set

Note: Inappropriate management of samples may result in errors during analysis. Written procedures should be followed to avoid such risks.

11.1 Sample management in the laboratory (including the receipt and preparation of samples) should be considered an important aspect in good chromatography practices.

11.2 Samples received for analysis should be entered in an appropriate record that ensures the traceability of the sample detail and analysis.

11.3 Samples should be stored under appropriate conditions.

11.4 Samples (as well as blank and standard solutions) should be prepared in accordance with the authorized specifications and standard test procedures. Records for the preparation should be maintained.

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

11.6 Standard and sample solutions prepared for use in chromatography should be used within defined timelines derived from analytical procedure validation and stability data, as appropriate.

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

11.8 Where carry-over or interference in analysis is relevant, suitable precautions should be taken, such as the inclusion of a blank in the sequence of injections.

11.9 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 this is done, authorized procedures should clearly describe this approach. (Normally, only standard solutions may be used for this purpose, unless otherwise needed and justified e.g. biologics). The electronic record of results in such cases should be saved and stored, together with the results of the sample set for analysis.

11.10 A system suitability test (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. The SST should meet the predefined acceptance criteria, before samples are injected and throughout the analysis.

11.11 Acceptance criteria should be set for the 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.

11.12 Bracketing standards (standard solution injections) should be included in the sample set, at defined intervals, where appropriate. The number of bracketing standards included in a sample set should be defined. Compliance with the defined acceptance criteria should be verified.

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

12. Chromatographic methods (acquisition and processing)

12.1 Chromatographic methods should be suitable for their intended use. Appropriate acceptance criteria should be specified for parameters such as selectivity (resolution and/or peak-to-valley ratio), sensitivity (signal-to-noise ratio), peak symmetry, repeatability and integration conditions (if applicable).

12.2 Where non-pharmacopoeia methods are to be used, these should be developed, validated and described in detail in standard procedures. These procedures should be followed by qualified, trained, experienced personnel.

12.3 It is preferable that methods are created and saved in the chromatographic system by authorized personnel. The method selected for analysis from the saved methods should not be modified, unless approved for the intended purpose by authorized personnel.

12.4 Data acquisition and processing software should be appropriately validated or verified as being suitable for use. Methods selected for acquisition and processing should be traceable and reflected in the audit trail.

12.5 Methods should be proven to remain in a validated state throughout their life-cycle.

12.6 Chromatographic conditions (such as the composition of the mobile phase, pH, column dimensions) may be adjusted, within specified limits and in accordance with written procedures, to obtain the separation required. The adjustments made should be within the limits specified (such as defined in the design space of the analytical procedure). The SST requirements (e.g. resolution, symmetry, repeatability) should be met, and retention times and relative retention should be similar.

13. Peak integration

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

13.2 Where possible, HPLC and GC instruments should be interfaced with computerized chromatographic data-capturing and processing systems that are capable of applying the integration parameters set, automatically and consistently.

13.3 To facilitate the accurate integration of chromatographic peaks, it is preferable that all of the peaks are fully separated. However, when quantitative data are to be obtained from unresolved peaks, the laboratory should have clear policies as to how such peaks should be integrated. This should include a description of the type of integration to be used, with a justification for its use, including, for example:

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

13.4 Validated methods, specified chromatographic conditions and good chromatography practices should facilitate obtaining symmetrical peaks. Where atypical peak shapes are observed, these should be investigated and appropriate action taken.

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

13.6 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 trained, experienced users should be granted privileges to do so. Records and justification should be given when this procedure is followed.

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

14. Data management

14.1 Chromatographic data should be managed in accordance with this guideline and other related guidelines (*1*–*3*).

14.2 Procedures should be followed for timely processing and review of data and reporting of results.

14.3 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.

14.4 Data should be safely stored in a way that includes control over access to data. Backed-up data should be stored at a separate location. Some data should be randomly selected for restoration and verification, at defined intervals, in accordance with a written procedure.

14.5 Where appropriate, paper printed records (including data and metadata) may be retained as part of the analytical report reflecting analyses performed.

14.6 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.

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

Note: See other guidelines addressing computerized systems (*1*), data integrity (*2*) and good documentation practices (*3*).

References

1. Good manufacturing practices: guidelines on validation. Appendix 5. Validation of computerized systems. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: fifty-third report. Geneva: World Health Organization; 2019: Annex 3 (WHO Technical Report Series, No. 1019; https://www.who.int/medicines/areas/quality_safety/quality_assurance/WHO_TRS_1019_Annex3.pdf?ua=1, accessed 29 January 2020).
2. Guideline on data integrity. Draft for comments. Geneva: World Health Organization; 2019 (Working document QAS/19.819; https://www.who.int/medicines/areas/quality_safety/quality_assurance/QAS19_819_data_integrity.pdf?ua=1, accessed 5 December 2019).
3. Guidance on good data and record management practices. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: fiftieth report. Geneva: World Health Organization; 2016: Annex 5 (WHO Technical Report Series, No. 996; https://www.who.int/medicines/publications/pharmprep/WHO_TRS_996_annex05.pdf, accessed 4 November 2019).

Further reading

- The International Pharmacopoeia, 9th ed. Geneva: World Health Organization; 2019 (https://apps.who.int/phint/en/p/docf/, accessed 4 November 2019).
- European pharmacopoeia (Ph. Eur.), 9th edition. Strasbourg: Council of Europe; 2019 (https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition, accessed 5 December 2019).
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- Good manufacturing practices: guidelines on validation. Appendix 6. Guidelines on qualification. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: fifty-third report. Geneva: World Health Organization; 2019: Annex 3 (WHO Technical Report Series, No. 1019; https://www.who.int/medicines/areas/quality_safety/quality_assurance/WHO_TRS_1019_Annex3.pdf?ua=1, accessed 29 January 2020).
- WHO good practices for pharmaceutical quality control laboratories. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-fourth report. Geneva: World Health Organization; 2010: Annex 1 (WHO Technical Report Series, No. 957; http://apps.who.int/medicinedocs/documents/s18681en/s18681en.pdf, accessed 5 December 2019).