GOOD CHROMATOGRAPHY PRACTICES
(July 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 Claire Vogel (vogelc@who.int) by 20 September 2019.

Working documents are sent out electronically and they will also be placed on the WHO Medicines website (http://www.who.int/medicines/areas/quality_safety/quality_assurance/guidelines/en/) for comments under the “Current projects” link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

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## SCHEDULE FOR DRAFT WORKING DOCUMENT QAS/19.791:

### GOOD CHROMATOGRAPHY PRACTICES

<table>
<thead>
<tr>
<th>Description of Activity</th>
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<tr>
<td>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.</td>
<td>October 2018</td>
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<tr>
<td>Preparation of first draft working document.</td>
<td>December 2018 - February 2019</td>
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<tr>
<td>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.</td>
<td>February – May 2019</td>
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<tr>
<td>Consolidation of comments received and review of feedbacks. Preparation of working document for discussion.</td>
<td>June 2019</td>
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<tr>
<td>Discussion of working document and feedbacks received during the informal Consultation on Good Practices for Health Products Manufacture and Inspection.</td>
<td>2-5 July 2019</td>
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<tr>
<td>Revision of the working document based on comments received during the informal Consultation on Good Practices for Health Products Manufacture and Inspection.</td>
<td>July 2019</td>
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<tr>
<td>Mailing of revised working document to the EAP inviting comments and posting the working document on the WHO website for public consultation.</td>
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<tr>
<td>Consolidation of comments received and review of feedbacks. Preparation of working document for discussion.</td>
<td>End of September 2019</td>
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<tr>
<td>Presentation to the Fifty-fourth meeting of the ECSPP.</td>
<td>14 -18 October 2019</td>
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<td>Any other follow-up action as required.</td>
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GOOD CHROMATOGRAPHY PRACTICES

1. INTRODUCTION AND SCOPE

The use of chromatography methods such as High-Performance 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 document.

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 (TLC) methods are also used, this approach is not specifically addressed in this document.)

Due 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).

This document provides information on good practices 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.

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.

audit trail
The audit trail is a form of metadata that contains information associated with actions that relate to the creation, modification or deletion of good practices (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 the 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.

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 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
Data integrity is the degree to which data are complete, consistent, accurate, trustworthy and reliable and that 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.

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.
**Exponential skim**

The creation of a curvature in the skim line in an attempt to approximate the underlying baseline of 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. Chromatographic systems should meet regulatory requirements and expectations for GXP. This should include, for example, ensuring that data are acquired, processed and stored in accordance with national legislation and ALCOA+ principles.

3.2. 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 and installed, should be appropriate for their intended use.

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). (See also the *WHO GMP: Guidelines on validation, Appendix 5: validation of computerized systems and Appendix 6: Guidelines on qualification, Annex 3, TRS 1019, 2019*).
4.4. Validation and qualification should be described in protocols and recorded in reports. Reports should contain documented evidence and include, for example, screen shots, 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 out of calibration or not meeting the predefined limits. Appropriate corrective and preventive action (CAPA) 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.
5.5. Manual records of user groups, users and their privileges should be concordant with electronic data.

6. **AUDIT TRAIL**

6.1. Chromatographic systems should have an audit trail(s) which reflects, for example, users, dates, times, original data and results, changes and reasons for change. (See also **WHO Guidance on good data and record management practices, TRS 996, Annex 5, 2016.**)

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 review of an audit trail (for example, each sample sequence or sample set in chromatographic analysis) and of the periodic review of audit trails. (Periodic review should be done at specified intervals where a random selection of audit trails are verified and may include system audit trail, changes in user privileges and other activities tracked in 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 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 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 their 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 is 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.

8.5. 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, procedures and pharmacopoeia. 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. Mobile phases should be filtered and degassed as 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. Tubing and fittings should be appropriate to ensure that the system performs as expected.

10.5. Column efficiency (number of theoretical plates) should be assured to ensure good chromatography.

10.6. Equilibration of columns, as well as controlling temperature and mobile phase during analysis, should be done when specified.

10.7. The required flow rate should be specified in relevant test procedures; and 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, flow rate and time.
10.10. Columns should be stored in a manner that ensures that they are not damaged.

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

11. SAMPLE MANAGEMENT

11.1. Sample management (including the receiving 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 which 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 time lines derived from analytical procedure validation and stability data as appropriate.

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

11.8. The sample set (sample sequence) 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.
11.9. 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 this is done, authorized procedures should clearly describe this approach and specify that only standard solutions may be used for this purpose. The electronic record of results in such cases should be saved and stored together with the results of the sample set for analysis.

11.11. 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 pre-defined acceptance criteria.

11.12. 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.

11.13. 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.

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

12. CHROMATOGRAPHIC METHODS (ACQUISITION AND PROCESSING)

12.1. Chromatographic methods should be suitable for their intended use. Appropriate values should be specified for parameters such as, slope sensitivity, noise threshold, peak width, area threshold, bunching factor and skim ratio.
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. Results acquired should be processed by validated methods. Methods for acquisition and processing selected 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.

13. CHROMATOGRAPHIC PEAKS

13.1. Chromatographic analysis should be done in accordance with procedures which 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+.

13.2. In addition to parameters such as accuracy, precision and linearity (see *WHO Guideline on Validation of Analytical Methods*), the following factors should also be considered during analytical method validation and appropriately applied during sample analysis:

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

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

14. PEAK INTEGRATION

14.1. Peak areas in 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 applying integration parameters set, automatically and consistently.

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. 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.
14.5. 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.

14.6. 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.

14.7. 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.

14.8. 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 (such as in chromatography) 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 (results and metadata) should be managed in accordance with these guidelines and other relevant guidelines relating to cleaning validation, chromatography, data integrity 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 should be managed in accordance with this guideline and other related guidelines such as Good Data and Record Management.

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

<table>
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<tr>
<th>Acronym</th>
<th>Definition</th>
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<td>ALCOA</td>
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<td>CAPA</td>
<td>corrective and preventive action</td>
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<td>DQ</td>
<td>design qualification</td>
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<td>EDMS</td>
<td>Electronic Data Management Systems</td>
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<td>FAT</td>
<td>factory acceptance test</td>
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<td>GC</td>
<td>gas chromatography</td>
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<td>GMP</td>
<td>good manufacturing practices</td>
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<td>GXP</td>
<td>good practices</td>
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<td>HPLC</td>
<td>High Pressure Liquid Chromatography</td>
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<td>ID</td>
<td>operator identification</td>
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Further reading


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