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QUALIFICATION OF EQUIPMENT

QUALIFICATION OF ANALYTICAL COLUMNS

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ANNEX 11 OF THE OMCL NETWORK GUIDELINE
"QUALIFICATION OF EQUIPMENT"

QUALIFICATION OF ANALYTICAL COLUMNS

Note: Mandatory requirements in this guideline and its annexes are defined using the terms "shall" or "must". The use of "should" indicates a recommendation. For these parts of the text other appropriately justified approaches are acceptable. The term "can" indicates a possibility or an example with non-binding character.

Contents

1. INTRODUCTION

The present document outlines the general principles for the qualification of analytical columns used within OMCLs. Analytical columns are considered to be equipment, and as an essential part of the chromatographic system (Ph. Eur. Chapters 2.2.28, 2.2.29), they can influence the results obtained (ISO/IEC 17025:2017, Chapter 6.4 Equipment, point 6.4.1). For this reason, the qualification of analytical columns should be carried out whenever considered necessary by the OMCL.

The OMCL is responsible for defining a suitable qualification scheme for use during the life cycle of the column, depending, for example, on the intended purpose and the frequency of use.

2. SCOPE

This document describes the requirements and general criteria for the qualification of analytical columns used in Liquid Chromatography (LC, UHPLC) and Gas Chromato-graphy (GC) procedures.

It can be used as guidance by OMCLs when planning, performing and documenting the qualification process, upon receipt and during the life cycle of analytical columns.

Exemplary procedures provided in Annexes 1 and 2 have non-binding character. They can be helpful when carrying out the required qualification. Nevertheless, it is left to the professional judgment and background experience of each OMCL to apply the most suitable procedure, in order to prove that the analytical columns are suitable for their intended use.

3. GLOSSARY AND DEFINITIONS

The terminology used in this document is consistent with that used in Ph. Eur. Chapter 2.2.46 “Chromatographic separation techniques”.

4. CONSIDERATIONS FOR QUALIFICATION UPON PURCHASING

Incoming columns shall be verified for physical damage due to shipping, for compliance with the purchase order and for completeness of the documentation.

The OMCL shall retain records related to the management of columns for the applicable requirements described in ISO/IEC 17025:2017 clause 6.4.13.

5. INITIAL QUALIFICATION

The analytical column shall be verified upon receipt or before the first use by checking the documentation (e.g. certificate of analysis) or by performing qualification of the column according to a pre-defined procedure.

Verification/qualification shall be documented (including, e.g. date of verification/qualification, reference to the procedure for qualification, reference materials or reagents used, results, chromatographic equipment used, pass/fail conclusion, etc.). This record can be used to make a complaint in cases where the column does not comply with the manufacturer’s specification.

5.1 Qualification parameters

It is the responsibility of the OMCL to choose the most appropriate procedure, parameters and acceptance criteria. These should be appropriate to the intended use of the column. These parameters can include resolution, symmetry factor, theoretical plates, etc. Some typical limits are given as examples in Annexes 1 and 2.

6. PERIODICAL QUALIFICATION

The qualification of the columns can be performed periodically using the same test compounds/procedure. A systematic record of the qualification results should be maintained in the laboratory.

6.1 Qualification procedure

At least one of the procedures chosen for initial qualification can be applied for periodical qualification. The first chromatogram obtained with a new column in the laboratory can serve as a reference of column performance at the initial time point and used as a reference for subsequent verifications. Slight variations can be observed on chromatograms due to different LC or GC configuration of the equipment (e.g. dead volumes), operating environment, system electronics, etc. These can be taken into consideration whenever the acceptance criteria are set in-house.

6.2 Frequency of qualification

The laboratory can define and document the frequency of qualification whenever periodic qualification is considered appropriate and useful. The frequency depends on the use of the column (e.g. routine or “spot” analysis). For instance, a qualification according to section 5 (above) could be performed at fixed time intervals (e.g. once a year) or depending on the extent of use, e.g. after a defined number of injections.

6.3 Qualification records

A record of the qualification results can help the user to monitor the column performance over time and inform the decision to discard it. A column is considered suitable for the intended use when the acceptance/system suitability criteria are met.

7. IN-USE QUALIFICATION

In-use qualification corresponds to the system suitability test for the method used. It shall be performed on a regular basis before each test and/or during the sequence of injections. In cases where the system suitability criteria are not met, the qualification of the column can be performed, to verify the consistency with the initial performance.

The analytical procedure (e.g. mobile phase used) and the number of samples injected can contribute to the degradation of the stationary phase. The information related to the tested samples and column usage/storage conditions shall be traceable in the records (for example: samples analysed and matrix for finished products, chromatography conditions (mobile phase, temperature, pH value…).

8. PROCEDURES FOR QUALIFICATION OF ANALYTICAL COLUMNS

Qualification of column performance can be done using one or more of the following approaches:

a) The column can be tested under the same conditions described in the quality control leaflet provided by the manufacturer. The same parameters should be checked and the results should be compared with the manufacturer's results. Alternatively, other test mixtures may be used.

b) If the column is used only for specific test methods, it might be appropriate to perform the qualification with the system suitability test described for the test method.

c) Columns with similar stationary phases can be qualified using a general test mixture/procedure.

Most of the examples given below in Annexes 1 and 2 are qualification procedures provided by manufacturers.

The parameters tested for the qualification of the column should be calculated and reported as defined by Ph. Eur. Chapter 2.2.46. and the results used for verification of the column performance.

Consider any differences that might occur between the parameters defined in the manufacturer specification or instrument default calculations and the requirements of Ph. Eur. Chapter 2.2.46. (Note: the tailing factor calculated by the instrument software is quite often USP tailing by default).

9. REFERENCES

1) ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
2) Ph. Eur. Chapter 2.2.28. "Gas chromatography".
3) Ph. Eur. Chapter 2.2.29 "Liquid chromatography".
4) Ph. Eur. Chapter 2.2.46 "Chromatographic separation techniques".
5) Column performance test mixture for liquid chromatography - standard reference material 870. NIST.
6) HPLC column classification. USP Pharmacopeia Forum, Vol. 31(2).

APPENDIX 1

TABLE 1: EXAMPLES FOR QUALIFICATION OF ANALYTICAL COLUMNS FOR LC

The chromatographic conditions provided below apply to a column size of 250 mm (length) × 4.6 mm (diameter). These examples are also applicable for qualification of superficially porous particle (SPP) columns. When a column with other dimensions or SPP is used, the chromatographic conditions should be adjusted according to Ph. Eur. chapter 2.2.46.

TYPE of COLUMN	***Chromatographic settings:***	***Test mixture of: (solvent mobile phase)***	***Acceptance criteria***
RP-LC C-8 and C-18 (also appropriate for C-4).	Mobile phase: acetonitrile:water = 58:42 v/v % Flow: 1.0 mL/min Detection: 254 nm Temperature: room temperature Injection volume: 5 µL	Uracil: 0.005 mg/mL Toluene: 4.0 mg/mL Phenol: 0.7 mg/mL N, N-diethyl-m-toluamide: 0.6 mg/mL	A_s = 0.8 to 1.5 R_s > 1.5 (between adjacent peaks) N (toluene) > 3000 $\alpha_{phenol/toluene}$ and $\alpha_{N,N\text{-}diethyl\text{-}m\text{-}toluamide/toluene}$ ≥ 1.5 Uracil - indicator for the hold-up time (t_M)
RP-LC CN	Mobile phase: methanol:water = 70:30 v/v % Flow: 1.0 mL/min Detection: 254 nm Temperature: room temperature Injection volume: 5 µL	Uracil: 10 µg/mL Phenol: 200 µg/mL Toluene: 800 µg/mL 4-Cl-nitrobenzene: 25 µg/mL Naphthalene: 40 µg/mL	A_s = 0.8 to 1.5 N (Naphthalene) > 3000 R_s ≥ 1.5 (between adjacent peaks) Uracil - indicator for the hold-up time (t_M)
RP-LC PHENYL (phenyl propyl and phenyl hexyl)	Mobile phase: acetonitrile:water = 65:35 v/v % Flow: 1.0 mL/min Detection: 254 nm Temperature: room temperature Injection volume: 1 µL	Uracil: 0.01 mg/mL Acetophenone: 0.22 mg/mL Toluene: 9.42 mg/mL Naphthalene: 9.42 mg/mL	A_s = 0.8 to 1.5 R_s > 1.5 (between adjacent peaks) N (Naphthalene) > 3000 $\alpha_{acetophenone/toluene}$ and $\alpha_{toluene/naphthalene}$ ≥ 1.5 Uracil - indicator for the hold-up time (t_M)
NP-LC (for Si, NH_2, NO_2, Diol, CN)	Mobile phase: hexane:ethanol R = 95:5 v/v % Flow: 1.0 mL/min Detection: 254 nm Temperature: room temperature Injection volume: 5 µL	Toluene: 1.0 mg/mL Diethyl phthalate: 1.0 mg/mL Dimethyl phthalate: 1.0 mg/mL	A_s = 0.8 to 1.5 R_s > 1.5 (between adjacent peaks) N > 3000 $\alpha_{diethyl\ phthalate/dimethyl\ phthalate}$ ≥ 1.5 Toluene - indicator for the hold-up time (t_M)
STRONG AND WEAK CATION-EXCHANGE (SCX; WCX)	Mobile phase: 0.15 M di-ammonium hydrogen phosphate buffer pH=6.0 Flow: 1.0 mL/min Detection: 254 nm Temperature: room temperature Injection volume: 10 µL	Uracil: 7 µg/mL Cytosine: 7 µg/mL (solvent: water)	A_s = 0.8 to 1.5 N > 3000 R_s > 1.5 $\alpha_{uracil/cytosine}$ ≥ 1.5

TYPE of COLUMN	***Chromatographic settings:***	***Test mixture of: (solvent mobile phase)***	***Acceptance criteria***
STRONG AND WEAK ANION-EXCHANGE COLUMNS (SAX; WAX)	Mobile phase: 0.05 M sodium di-hydrogen phosphate buffer pH=3.0 Flow: 1.0 mL/min Detection: 254 nm Temperature: room temperature Injection volume: 10 µL	Uridine: 7 µg/mL Uridine monophosphate: 7 µg/mL	A_s = 0.8 to 1.5 N > 3000 R_s > 1.5 $\alpha_{uridine/uridine\ monophosphate} \geq 1.5$
QUALIFICATION OF CHIRAL COLUMNS		It is recommended to test this column type with a test mixture according to the QC certificate of the manufacturer.	Results should be compared with those in the QC chromatogram of the manufacturer.
SIZE-EXCLUSION COLUMNS A very widely-used column in the quality control of therapeutic proteins (e.g. immunoglobulin monographs *0338* and *0918* and albumin monograph *0255*) is based on hydrophilic silica gel for chromatography R with a fractionation range for protein of 10000 to 500000.	Mobile phase: dissolve 4.873 g disodium hydrogen phosphate dihydrate R, 1.741 g sodium dihydrogen phosphate monohydrate R and 11.688 g sodium chloride R in 1 L of water R. Column dimensions: - 10 µm particle size: l = 600 mm, I.D.: 7.5 mm - 5 µm particle size: l = 300 mm, I.D.: 7.5 mm Flow rate: 0.5 mL/min Detection: 280 nm Inj. Volume: 20 µL	Thyroglobulin (bovine, MW 670,000): 5.0 g/L γ-Globulin (bovine, MW 158,000): 5.0 g/L Ovalbumin (chicken, MW 44,000): 5.0 g/L Myoglobin (horse, MW 17,000): 2.5 g/L Vitamin B12 (MW 1,350): 0.5 g/L	The elution order is Thyroglobulin, γ-Globulin, Ovalbumin, Myoglobin and Vitamin B12. There may be additional peaks between Thyroglobulin and γ-Globulin (γ-Globulin-dimer peak) and in front of thyroglobulin (aggregates peak). N (Vitamin B12): NLT 20,000 A_s (Vitamin B12): 0.8 to 1.5 R_s (Myoglobin/Ovalbumin): NLT 2.5

APPENDIX 2

TABLE 1: EXAMPLES FOR THE QUALIFICATION OF ANALYTICAL COLUMNS FOR GAS CHROMATOGRAPHY

TYPE of COLUMN	***Chromatographic settings* (FID detector)*:***	***Test mixture of:*** ***(solvent: methylene chloride)***	***Acceptance criteria***
NON-POLAR	*Narrow-bore columns (0.25 or 0.32 mm ID):* Carrier gas: Helium Linear gas rate: 20-25 cm/sec Injection port temperature: 220°C Oven temperature: 100-135°C FID temperature: 220°C Split ratio: 100:1 (5 ng of each component is delivered onto the column) Injection volume: 1 µL *Wide-bore capillary columns (0.53 or 0.75 mm ID):* Dilute the mixture 1:10 in methylene chloride and inject 0.2 µL onto the column in direct injection mode.	2-octanone: 0.5 mg/mL Decane: 0.5 mg/mL 1-octanol: 0.5 mg/mL 2,6-dimethyl-phenole: 0.5 mg/mL Undecane: 0.5 mg/mL 2,6-dimethylaniline: 0.5 mg/mL Dodecane: 0.5 mg/mL Tridecane: 0.5 mg/mL	A_s = 0.8 to 1.5 N/metre (peak with k between 5 and 7)* α (between adjacent peaks) ≥ 1.5 Methane -indicator for the hold-up time (t_M)
MEDIUM POLAR	*Narrow bore columns (0.25 or 0.32 mm ID):* Carrier gas: Helium Linear gas rate: 20-25 cm/sec Injection port temperature: 220°C Oven temperature: 100-135°C FID temperature: 220°C Split ratio: 100:1 (5 ng of each component is delivered onto the column) Injection volume: 1 µL *Wide-bore capillary columns (0.53 or 0.75 mm ID):* Dilute the mixture 1:10 with methylene chloride and inject 0.2 µL onto the column in direct injection mode.	N-decane: 0.5 mg/mL N-dodecane: 0.5 mg/mL N-tetradecane: 0.5 mg/mL N-tridecane: 0.5 mg/mL N-undecane: 0.5 mg/mL 1-octanol: 0.5 mg/mL 2-octanone: 0.5 mg/mL 2,6-dimethylaniline: 0.5 mg/mL 2.6-dimethyl-phenole: 0.5 mg/mL	A_s = 0.8 to 1.5 N/metre (peak with k between 5 and 7)* α (between adjacent peaks) ≥ 1.5 Methane -indicator for the hold-up time (t_M)

TYPE of COLUMN	***Chromatographic settings* (FID detector)*:***	***Test mixture of:*** ***(solvent: methylene chloride)***	***Acceptance criteria***
POLAR	*Narrow-bore columns (0.25 or 0.32 mm ID):* Carrier gas: Helium Linear gas rate: 20-25 cm/sec Injection port temperature: 220°C Oven temperature: 145-185°C FID temperature: 220°C Split ratio: 100:1 (5 ng of each component is delivered onto the column) Injection volume: 1 µL *Wide-bore capillary columns (0.53 or 0.75 mm ID):* Dilute the mixture 1:10 with methylene chloride and inject 0.2 µL onto the column in direct injection mode.	N-eicosane: 0.5 mg/mL N-heptadecane: 0.5 mg/mL N-hexadecane: 0.5 mg/mL N-octadecane: 0.5 mg/mL N-pentadecane: 0.5 mg/mL 1-octanol: 0.5 mg/mL 2-octanone: 0.5 mg/mL 2,6-dimethylaniline: 0.5 mg/mL 2,6-dimethyl-phenole: 0.5 mg/mL	A_s = 0.8 to 1.5 N/metre (peak with k between 5 and 7)* α (between adjacent peaks) ≥ 1.5 Methane -indicator for the hold-up time (t_M)

*requirements are given in Table 2

LEGEND:
A_s: Symmetry factor
R_s: Resolution
N: Plate number
α: selectivity factor (**α**) i.e. ratio of the retention factors (k) of the two adjacent peaks

Plate number

The column performance may be calculated for a given component as the plate number (also referred to as number of theoretical plates).
This parameter is very useful for comparisons of GC columns. Table 2 below represents the requirements for the average column performance, given in plates/metre, according to the internal diameter and polarity of the phase.

Table 2. Comparison of average efficiency by phase polarity and internal diameter.

Internal diameter	Polarity of the column		
	Non-polar	Intermediate polarity	Polar
0.1 mm	10000	-	-
0.2 mm	4500	4200	4000
0.32 mm	3200	3000	2500
0.53 mm	1500	1350	1300