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QUALIFICATION OF ANALYTICAL COLUMNS

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QUALIFICATION OF ANALYTICAL COLUMNS

1. INTRODUCTION

The present document outlines the general principles for the qualification of analytical columns used within OMCLs. It has been agreed that analytical columns represent consumables, but are also an essential part of the chromatographic equipment (Ph. Eur. 2.2.28, 2.2.29).
For this reason, qualification of analytical columns is considered necessary. The purpose of this document is to give general requirements for the qualification of different types of analytical columns. Actually, due to the great variety of analytical columns, it is not possible to provide specific requirements for all of them. The levels of qualification mentioned in this document refer to the definitions given in OMCL Guideline PA/PH/OMCL (08) 73 2R.

This document may be used as a guide to OMCLs for planning, performing and documenting the qualification process upon receipt and usage of analytical columns. It represents a recommendation document.

2. AIM AND SCOPE OF THE DOCUMENT

This document describes the requirements for the qualification of analytical columns used in LC and GC. The aim is to provide general criteria for qualification and examples for testing the most commonly used analytical columns. Other approaches may be used.

3. CONSIDERATIONS FOR LEVEL I QUALIFICATION

Although there is no need for a formal level I procedure, criteria used for the ordering of columns should comply with the special needs of the intended test procedure.

4. LEVEL II QUALIFICATION

At Level II of the qualification of analytical columns, it is recommended to check the column for physical damage which may appear during shipping, to verify that the column received complies with the order and that the supplier has submitted all necessary documents and information.

The analytical column may be appropriately tested before use by the OMCL according to a pre-defined procedure. This testing procedure may be performed on receipt or, at the latest, prior to first use and should be documented in a suitable qualification record (see Annex 2).
After the qualification, the laboratory should record the results.
The OMCL should implement a system for identification of available columns and should maintain a list of all columns in the laboratory.
The type of qualification depends on the intended use of the column and can follow different approaches. Future re-qualifications should follow the same test procedures as used in level II qualification.
Verification of column performance can be done by one or more of the following approaches:
a) By testing the column under the same conditions given in the original quality control chromatogram of the manufacturer. The same parameters have to be checked and the

results have to be compared with the results of the manufacturer. Alternatively, other test mixtures may be used.
b) If the column will be used only for specific test methods, it might be appropriate to perform the qualification with an appropriate system suitability test that is specific for the test procedure.
c) Alternatively, a common test mixture for columns with similar stationary phases may be used.

5. LEVEL III QUALIFICATION

The OMCL should ensure that the column is under quality control and column verification may be performed periodically using the same test compounds. A systematic record of the results has to be maintained in the laboratory. The qualification may be done as described in Chapter 4. At least one of the approaches chosen for level II qualification should be followed in level III qualification.

The first chromatogram obtained on a new column in the laboratory may serve as a reference and defines column performance at time t = 0 in the OMCL. It should be used as the base criterion for subsequent verifications. Slight variation may be obtained on different LC or GC systems due to the quality of the connections, operating environment, system electronics, etc. These can be reflected in the acceptance criteria used in the qualification.

A record of the results helps the user to monitor column performance and to know when a column should be replaced. A column should be replaced whenever its performance falls outside expectations.

The type and number of samples analysed contributes to column degradation. The number and type of analyses performed should be traceable for each column. A typical example for recording could be the following form:

Column size:		Serial №:	
Stationary phase:		Unique №:	

Date	Active substance/s (INN)	Chromatography condition (mobile phase, temperature, carrier gas and other)	Storage condition:	Notes	Analyst (name and signature)

Qualification parameters:
It is the responsibility of the OMCL to choose the appropriate quality parameters and corresponding acceptance criteria. These should be appropriate to the intended use of the column. These parameters could include resolution, symmetry factor, theoretical plates, etc. Examples are given in chapters 6 and 7.

Frequency of qualification:
The laboratory should define and document the frequency of qualification depending on the use of the column. For instance, a qualification according to section 4.a (above) could be performed based on fixed time intervals (e.g. once a year) or, more individually, based on the frequency of use of a column, e.g. after a defined number of injections.

6. LEVEL IV QUALIFICATION

Level IV qualification corresponds to the system suitability test of the individual test procedure.

7. GLOSSARY

Terms and definitions:

Height of a theoretical plate (H)

The height of a theoretical plate of a component may by calculated using the following equation:

$H=\frac{L}{N}$, where

L is the length of the column [mm], and
N is the calculated number of theoretical plates of the component.
A good column has a large N and a small H.

Plate per meter
The efficiency of a chromatography column may be expressed for a given component by the number of theoretical plates per meter.
This parameter is used mainly in GC. It is very useful for comparisons of GC columns.
Table 1 below represents the requirements for the average column efficiency, given in plates/meter, according to the internal diameter and polarity of the phase.

Table 1. 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

Plate number (N) *– as specified in Ph. Eur. 2.2.46*
The plate number calculated from a non-retained peak is a measure of the column packing efficiency whereas, in the case of peaks eluted later, mass-transfer processes also contribute to the plate number. As a general rule, N is higher for retained compounds

because their band broadening by extra-column volumes (dead volumes) is lower than for the early eluted peaks. The plate number is a function of the column's dimensions (diameter, length and film thickness), the compound and its retention.

Qualification
Qualification is performed to assure that a given system, premises, equipment and column is able to meet pre-determined acceptance criteria.

Reduced plate height (h)
Information on the number of theoretical plates, or plate height, can cause problems due to mass transfer, so dimensionless values are much more suitable for absolute comparisons of a whole range of different types of columns (long, short, thick or thin). Reduced plate height is calculated as:

$$h=\frac{H}{d_p}, \text{ where}$$

H is the height of a theoretical plate, and
d_p is the particle diameter [µm].

Relative retention (r) *– as specified in Ph. Eur. 2.2.46.*

Resolution (R_s) *– as specified in Ph. Eur. 2.2.46.*

Retention time *– as specified in Ph. Eur. 2.2.46.*

Retention factor (k) *– as specified in Ph. Eur. 2.2.46*
The larger this value, the greater the amount of a given solute in the stationary phase and, hence, the greater the retention of the column.

Selectivity (α)
The chromatographic ability of the system to separate two compounds represents its selectivity.

$$\alpha=\frac{k_2}{k_1}, \text{ where}$$

k_2 and k_1 are retention factors of two compounds with $k_2 > k_1$.

Symmetry factor (A_s) *– as specified in Ph. Eur. 2.2.46.*

8. REFERENCES

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ANNEX 1:

A. EXAMPLES FOR QUALIFICATION OF ANALYTICAL COLUMNS FOR LC

The chromatographic conditions in the next test examples are for columns with different stationary phases and dimensions of 250 mm x 4.6 mm. When a column with other dimensions is tested, acceptance criteria and chromatographic conditions have to be adapted according to the requirements of Ph. Eur. 2.2.46. Most of the examples given below have been recommended by column manufacturers.

BACKGROUND INFORMATION ON CHARACTERISTICS OF RP LC-COLUMNS

The characteristics of the stationary phase are fundamentally dependent on their physico-chemical and chemical properties. Important properties include particle diameter, metal content, pore size, surface, the type of functional group on the surface, and also the density of the surface modifier (e.g. carbon load). In addition to these parameters, the various stationary phases available can also be differentiated based on their chromatographic properties.

The most important properties of reverse phase models are:

Hydrophobicity **–** refers to the property of a stationary phase to retain the hydrophobic compounds as much as possible. The strength of the retention capacity of an individual phase depends substantially on the surface modification; the longer the hydrocarbon chain, the greater the hydrophobicity. If the hydrocarbon chains are shorter, the analytes have less space for adsorbtion, leading to lower hydrophobicity.

Silanol activity **–** silanol groups have a large influence on the selectivity of an RP-HPLC. On the one hand, undesired secondary interactions result from the silanol groups, whereby the basic substances display tailing through interactions with the acidic silanol groups. On the other hand, such secondary interaction is actually required in some separations to get the desired selectivity. To minimise silanophilic activity, many stationary phases are end-capped.

Polar selectivity **–** refers to the property of a phase to undergo polar interaction with analytes. RP with alkyl groups shows weak polar selectivity.

QUALIFICATION OF RP-LC C-8 AND C-18 COLUMNS

The following test is appropriate for qualification of this type of column using a mixture of four compounds (uracil, toluene, phenol and N,N-Diethyl–m-toluamide), with a UV-Vis detector:

Chromatographic settings:
Mobile phase: acetonitrile:water = 58:42 v/v %
Flow: 1.0 ml/min
Detection: 254 nm
Temperature: room temperature
Injection volume: 5 µl

Solutions:
Prepare a mixture of four compounds in solvent acetonitrile:water = 58:42 v/v % with the following concentrations:
- Uracil – 0.005 mg/ml
- Toluene – 4.0 mg/ml
- Phenol – 0.7 mg/ml
- N, N-diethyl-m-toluamide – 0.6 mg/ml

Method:
After the system is equilibrated, inject the prepared mixture at least twice. The retention time, plate number and symmetry factor have to be recorded for all peaks. Calculate the retention factor (k), resolution between the adjacent peaks and reduced plate height (h). Calculate the selectivity for the two compound pairs phenol/toluene and N,N-diethyl-m-toluamide/toluene.

Criteria for characterisation and limits:
- The elution order is: Uracil, Phenol, N,N-diethyl-m-toluamide and Toluene.
- Uracil – this component is used as an indicator for the hold-up time (t_M), which is required to calculate the retention factor (k).
- Toluene - the retention of this component is a result of solvophobic interactions. Calculate the retention factor for this non-polar compound, which provides information on column strength. It is used to calculate column plate number (N) and reduced plate height (h).
- N,N-diethyl-m-toluamide – the calculated symmetry factor of this basic compound is used for the measurement of silanol activity. A more symmetrical peak is indicative of column deactivation. It is a polar basic analyte that is sensitive to phase loss and silanol activity. As end-capping is lost, peak shape and efficiency should suffer.
- Phenol – is a weak acid and, together with toluene and N,N-diethyl-m-toluamide, it is used for determination of the polar selectivity of the stationary phase.

- A_s = 0.8 to 1.5
- R_s > 1.5 (between adjacent peaks)
- The number of plates N for toluene > 3000
- $\alpha_{phenol/toluene}$ and $\alpha_{N,N\text{-diethyl-m-toluamide/toluene}} \geq 1.5$
- Reduced peak heights and retention factors are necessary for comparisons between columns from different manufacturers.

The conditions described above for testing C-8 and C-18 column are also appropriate for C-4 columns.

QUALIFICATION OF RP-LC CN COLUMNS

Cyano-phases have moderate hydrophobicity compared to alkyl ligands.
The cyano group contains strong dipoles that can interact with other dipoles in solutes and can separate polar molecules.
The following test is appropriate for qualification of this type of column, using a mixture of five compounds (uracil, phenol, toluene, 4-Cl-nitrobenzene and naphthalene), with a UV-Vis detector:

Chromatographic settings:
Mobile phase: methanol:water = 70:30 v/v %
Flow: 1.0 ml/min
Detection: 254 nm
Temperature: room temperature
Injection volume: 5 µl

Solutions:
Prepare a mixture of five compounds in solvent methanol:water = 70:30 v/v % with the following concentrations:
- Uracil – 10 µg/ml
- Phenol – 200 µg/ml
- Toluene – 800 µg/ml
- 4-Cl-nitrobenzene – 25 µg/ml
- Naphthalene – 40 µg/ml

Method:
After the system is equilibrated, inject the prepared mixture at least twice. The retention time, plate number, asymmetry and resolution between the adjacent peaks have to be recorded for all peaks. Calculate the retention factor (k) and reduced plate height (h).

Criteria for characterisation and limits:
- The elution order is: Uracil, Phenol, Toluene, 4-Cl-nitrobenzene and Naphthalene.
- Uracil – this component is used as an indicator for the hold-up time (t_M), which is required to calculate the retention factor (k).
- Toluene and Naphthalene - the retention of these components is a result of solvophobic interactions. The peaks can be used to calculate the column plate number (N) and reduced plate height (h).
- Phenol and 4-Cl-nitrobenzene – these polar compounds are used for determination of polar selectivity.

- A_s = 0.8 to 1.5
- The number of plates N for Naphthalene > 3000
- $R_s \geq 1.5$ (between adjacent peaks)
- Reduced peak heights and retention factors are necessary for comparisons between columns from different manufacturers

QUALIFICATION OF RP-LC PHENYL (phenyl propyl and phenyl hexyl) COLUMNS

The retention characteristics are similar to those of C-8 columns, but with different selectivity for polycyclic aromatic hydrocarbons.
Phenyl phases are π-basic (electron donating) and are similar in overall retention to alkyl phases. The alternate selectivity of phenyl phases is often explained by the π-π interactions available through the phenyl ring. Compounds that appear to exhibit differential selectivity on the phenyl phase include: small, water soluble molecules and peptides; π-acceptors; nitroaromatics; polar compounds; dipoles; heterocyclics; etc.
The following test is appropriate for qualification of this type of column, using a mixture of four compounds (uracil, acetophenone, toluene and naphthalene), with a UV-Vis detector:

Chromatographic settings:
Mobile phase: acetonitrile:water = 65:35 v/v %
Flow: 1.0 ml/min
Detection: 254 nm
Temperature: room temperature
Injection volume: 1 µl

Solutions:
Prepare a mixture of four compounds in solvent acetonitrile:water = 65:35 v/v % with the following concentrations:
- Uracil – 0.01 mg/ml
- Acetophenone – 0.22 mg/ml
- Toluene – 9.42 mg/ml
- Naphthalene – 9.42 mg/ml

Method:
After the system is equilibrated, inject the prepared mixture at least twice. The retention time, plate number, asymmetry and resolution between the adjacent peaks have to be recorded for all peaks. Calculate the retention factor (k) and reduced plate height (h). Calculate the selectivity for the two compound pairs acetophenone/toluene and toluene/naphtalene.

Criteria for characterisation and limits:
- The elution order is: Uracil, Acetophenone, Toluene and Naphthalene.
- Uracil – this component is used as an indicator for the hold-up time (t_M), which is required to calculate the retention factor (k).
- Naphthalene - the retention of this component is a result of solvophobic interactions. Calculate the retention factor for this non-polar compound, which provides information on column strength. It can be used for the calculation of column plate number (N) and reduced plate height (h).
- The calculated value of α for the compound pair acetophenone/toluene represents selectivity for specific molecular increments.
- The α value for the compound pair toluene/naphthalene represents selectivity for π-π interactions.

- A_s = 0.8 to 1.5
- R_s > 1.5 (between adjacent peaks)
- The number of plates N for Naphthalen > 3000
- $\alpha_{acetophenone/toluene}$ and $\alpha_{toluene/naphtalen} \geq 1.5$
- Reduced peak heights and retention factors are necessary for comparisons between columns from different manufacturers.

QUALIFICATION OF NP-LC COLUMNS (for Si, NH_2, NO_2, Diol, CN)

The following test is appropriate for qualification of this type of column, using a mixture of three compounds (Toluene, Diethyl phthalate and Dimethyl phthalate), with a UV-Vis detector:

Chromatographic settings:
Mobile phase: hexane:ethanol R = 95:5 v/v %
Flow: 1.0 ml/min
Detection: 254 nm

Temperature: room temperature
Injection volume: 5 µl

Solutions:
Prepare a mixture of three compounds in solvent hexane:ethanol R = 95:5 v/v %, with the following concentrations:
- Toluene – 1.0 mg/ml
- Diethyl phthalate – 1.0 mg/ml
- Dimethyl phthalate – 1.0 mg/ml

Method:
After the system is equilibrated, inject the prepared mixture at least twice. The retention time, plate number, asymmetry and resolution between the adjacent peaks have to be recorded for all peaks. Calculate the retention factor (k) and reduced plate height (h). Calculate the selectivity for the compound pair diethyl phthalate/dimethyl phthalate.

Criteria for characterisation and limits:
- The elution order is: Toluene, Diethyl phthalate and Dimethyl phthalate.
- Toluene – this component is used as an indicator for the hold-up time (t_M), which is required to calculate the retention factor (k).
- Diethyl phthalate and dimethyl phthalate - the retention of these components is the result of hydrophylic interactions. Calculate the retention factor for these polar compounds, which provides information on column strength. Plate number and peak asymmetry can be assessed using either of the phthalate peaks.

- A_s = 0.8 to 1.5
- R_s > 1.5 (between adjacent peaks)
- The number of plates N > 3000
- $\alpha_{\text{diethyl phthalate/dimethyl phthalate}} \geq 1.5$
- Reduced peak heights and retention factors are necessary for comparisons between columns from different manufacturers.

QUALIFICATION OF STRONG AND WEAK ION-EXCHANGE COLUMNS (SCX; WCX; SAX; WAX)

The stationary phase has electric charges on its surface. A resin with SO_3^{2-} groups is a strong cation exchanger and a COO^- resin is a weak cation exchanger. An anion exchanger contains NR_3^+ (strong) or NR_2H^+ or NH_3^+ (weak) groups and forms bonds with negatively-charged anions.

1. Qualification of SCX columns
These types of columns are mainly used in separating basic, water-soluble compounds. The retention of basic substances is governed by both pH and ionic strength.
The following test is appropriate for qualification of this type of column, using a mixture of two compounds (Uracil and Cytosine) and a UV-Vis detector:

Chromatographic settings:
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

Solutions:
Prepare a mixture of two compounds in solvent water with the following concentrations:
- Uracil – 7 µg/ml
- Cytosine – 7 µg/ml

Method:
After the system is equilibrated, inject the prepared mixture at least twice. Record the retention time, symmetry, plate number, resolution and selectivity for the two peaks.

Limits:
- A_s = 0.8 to 1.5
- The number of plates N > 3000
- R_s > 1.5
- $\alpha_{uracil/cytosine} \geq 1.5$
- Reduced peak heights are necessary for comparisons between columns from different manufacturers.

2. Qualification of SAX columns
This type of column is mainly used for separating anionic analytes.
The following test is appropriate for qualification of this type of column, using a mixture of two compounds (Uridine and Uridine monophosphate) and a UV-Vis detector:

Chromatographic settings:
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

Solutions:
Prepare a mixture of two compounds in solvent water, with the following concentrations:
- Uridine – 7 µg/ml
- Uridine monophosphate – 7 µg/ml

Method:
After the system is equilibrated, inject the prepared mixture at least twice. Record the retention time, symmetry, plate number, resolution and selectivity for the two peaks.

Limits:
- A_s = 0.8 to 1.5
- The number of plates N > 3000
- R_s > 1.5
- $\alpha_{uridine/uridine\ monophosphate} \geq 1.5$
- Reduced peak heights are necessary for comparisons of columns from different manufacturers.

QUALIFICATION OF CHIRAL COLUMNS

It is recommended to test this column type with a test mixture according to the quality control certificate accompanying the column. After the test is completed, results should be compared with those in the QC chromatogram of the manufacturer.

SIZE-EXCLUSION COLUMNS

Due to their different pore-sizes, size-exclusion columns differ widely with respect to their fractionation range. It is therefore not possible to cover all types of size-exclusion columns with one set of test conditions and specifications. The qualification of a typical column type is presented here only as an example. 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.

Chromatographic settings:
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 = 600mm, i.D. : 7.5 mm
- 5 µm particle size: l = 300mm, i.D. : 7.5 mm

Flow rate: 0.5 mL/min
Detection: 280 nm
Inj. Volume: 20 µL

Solutions:
Dissolve the following compounds in the mobile phase:

- 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

Method:
After the system is equilibrated, inject the prepared mixture at least twice. The retention time, plate number, resolution and symmetry factor should be calculated and used as acceptance criteria.

Criteria for characterisation and limits:
- 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).

- Plate number (Vitamin B12):	NLT 20,000
- Symmetry factor (Vitamin B12):	0.8 to 1.5
- Resolution (Myoglobin/Ovalbumin):	NLT 2.5

Test mixtures and conditions can be used for several other stationary phases. Limits, however, have to be individually defined for every column type.

B. EXAMPLES FOR THE QUALIFICATION OF ANALYTICAL COLUMNS FOR GAS CHROMATOGRAPHY

Background Information:
The separation of the components of the test mixture and its peak shapes serve to monitor column efficiency and as diagnostic probes for adverse adsorptive effects and the acid/base properties of a column.
Alcohols and diols are commonly used to detect the presence of active silanol groups in the capillary column. Ketones and aldehydes show the presence of sites that interact with Lewis acids. A group of acidic or basic compounds is frequently used to determine how neutral a column is. Esters and n-paraffins are used to determine column efficiency.
Functional groups of the stationary phases are related to the polarity of the stationary phases. Polarity is a useful tool because it influences component separation. The components that co-elute on a non-polar column might separate on a polar column, or vice versa.
Most of the examples given below have been recommended by column manufacturers.

QUALIFICATION OF NON-POLAR COLUMNS

The following test is proposed for qualification of this type of column, using a mixture of ten compounds and an FID detector:

Chromatographic settings for 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

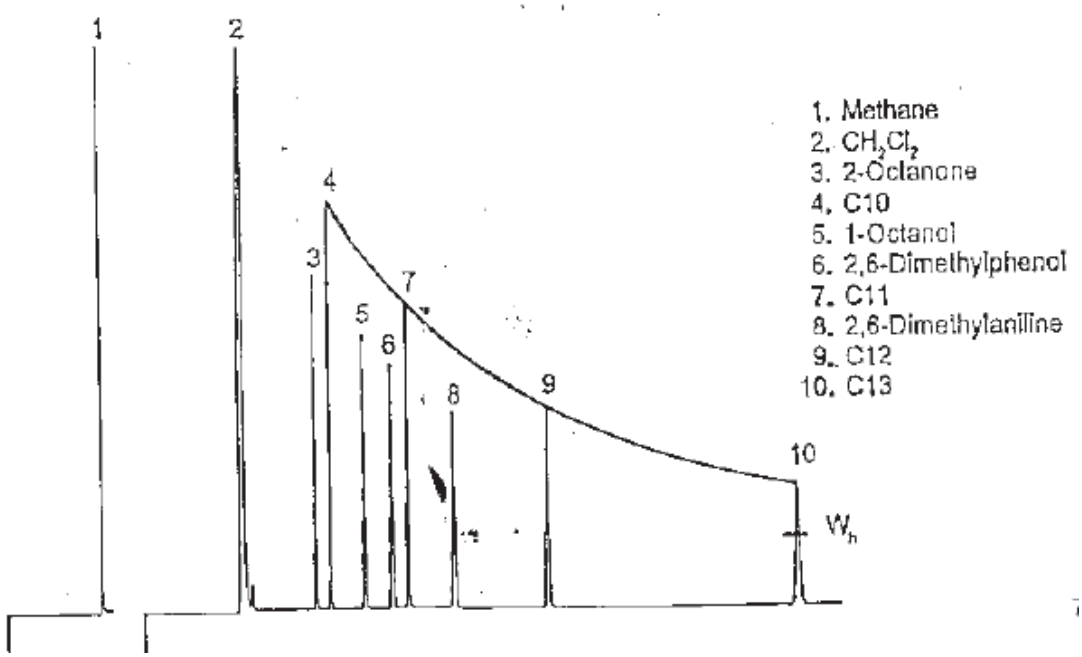
Chromatographic settings for wide-bore capillary columns (0.53 or 0.75 mm ID):
Dilute the mixture 1:10 in methylenchloride and inject 0.2 µl onto the column in direct injection mode.

Solutions:
Prepare a mixture of the eight compounds in solvent methylene chloride, with the following concentrations:
- 2-octanone – 0.5 mg/ml
- Decane – 0.5 mg/ml
- 1-octanol – 0.5 mg/ml
- 2,6-dimethylphenole – 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

Method:
After the system is equilibrated, inject the prepared mixture at least twice. The components in the test mixture provide important information about the column and system performance. Calculate the retention factor, selectivity for the adjacent peaks and plates per meter. Calculate the percentage of the peak height of n-alkanes by drawing a line connecting the peak maxima of these non-adsorbing peaks.

% response = (peak height of active component / height from baseline to curve connecting hydrocarbon peak apexes) x 100

The % response serves as an internal standard for accurately monitoring column performance for each active component. This technique includes all types of peak deformation.



Criteria for characterisation and limits:

- The elution order is: 2-octanone; N-decane; 1-octanol; 2,6-dimethylphenol; N-undecane; 2,6-dimethylaniline; N-dodecane and N-tridecane.
- Methane – this is used as an indicator for the hold-up time (t_M), which is required to calculate the retention factor (k) and mean linear gas velocity in the column ($\bar{u}$ = column length in cm/gas hold-up time in cm). Tailing of the peak indicates dead volume in the system.
- Calculate the retention factor (k) for all peaks.
- 2,6-dimethylaniline - tailing or reduced peak height for this component indicates that the active sites are acidic.
- 2,6-dimethylphenol – tailing or reduced peak heights for this component indicates that the active sites are basic.
- α for 2,6-dimethylaniline/2,6-dimethylphenol represents the acid/base ratio.
- 1-octanol - tailing or reduced peak heights indicate silanol or hydrogen-bonding sites.
- 2-octanol - tailing or reduced peak heights indicates Lewis acid sites.

- The last eluting hydrocarbons (with k between 5 and 7) are used for the calculation of the column plate number. Tailing of these peaks indicates dead volume, which can be caused by: broken columns, ferrule or glass fragments in columns, leaky connections, incomplete sweeping of the column outlet by the make-up gas or a damaged phase. A leading peak edge indicates overload, which can be caused by: too high a sample concentration, too low a split ratio, too low an oven temperature and too low a carrier gas velocity.

Limits:
- A_s = 0.8 to 1.5
- The number of plates per meter for the peak with k between 5 and 7: the requirements are given in Table 1 (see Chapter 7 above) according to the internal diameter and polarity
- α between adjacent peaks ≥ 1.5
- % response for active component ≥ 70%.

QUALIFICATION OF MEDIUM POLAR COLUMNS

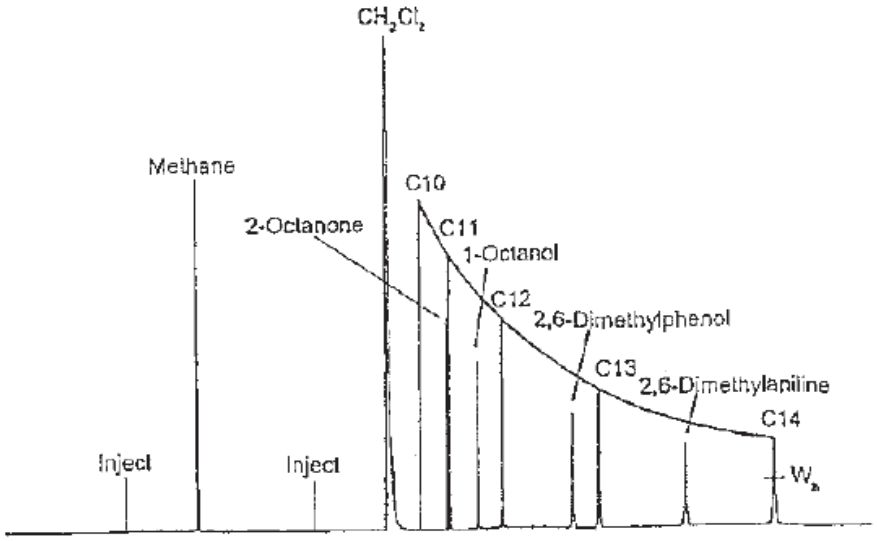
The following test is proposed for qualification of this type of column, using a mixture of nine compounds and an FID detector:

Chromatographic settings for narrow bore column (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

Chromatographic settings for wide-bore capillary columns (0.53 or 0.75 mm ID):
Dilute the mixture 1:10 with methylenchloride and inject 0.2 µl onto the column in direct injection mode.

Solutions:
Prepare a mixture of the nine compounds in solvent methylene chloride, with the following concentrations:
- 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-dimethylphenole – 0.5 mg/ml

Method:
After the system is equilibrated, inject the prepared mixture at least twice. Calculate the retention factor, selectivity for the adjacent peaks and plates per meter. Calculate the percentage of the peak height of n-alkanes by drawing a line connecting the peak maxima of these non-adsorbing peaks and calculate the % response for active compounds using the equation given above in the test for non-polar columns.



Criteria for characterisation and limits:

- The elution order is: N-decane ; 2-octanone; N-undecane; 1-octanol; N-dodecane; 2,6-dimethylphenol; N-tridecane; 2,6-dimethylaniline; N-tetradecane
- Methane – this component is used as an indicator for the hold-up time (t_M), which is required to calculate the retention factor (k) and mean linear gas velocity in the column ($\bar{u}$ = column length in cm/gas hold-up time in cm). Tailing of the peak indicates dead volume in the system.
- 2,6-dimethylaniline - tailing or reduced peak height for this component indicates that the active sites are acidic.
- 2, 6-dimethylphenol – tailing or reduced peak heights for this component indicates that the active sites are basic.
- α for 2,6-dimethylaniline/2,6-dimethylphenol represents the acid/base ratio.
- 1-octanol - tailing or reduced peak heights indicates silanol or hydrogen-bonding sites. It is a very sensitive probe for deactivation problems.
- 2-octanone - tailing or reduced peak heights indicate Lewis acid sites.
- The last eluting hydrocarbons (with k between 5 and 7) are used for the calculation of the column plate number.

Limits:
- A_s = 0.8 to 1.5
- The number of plates per meter for the peak with k between 5 and 7: the requirements are given in Table 1 (see Chapter 7 above) according to the internal diameter and polarity
- α between adjacent peaks ≥ 1.5
- % response for active component ≥ 70%.

QUALIFICATION OF POLAR COLUMNS

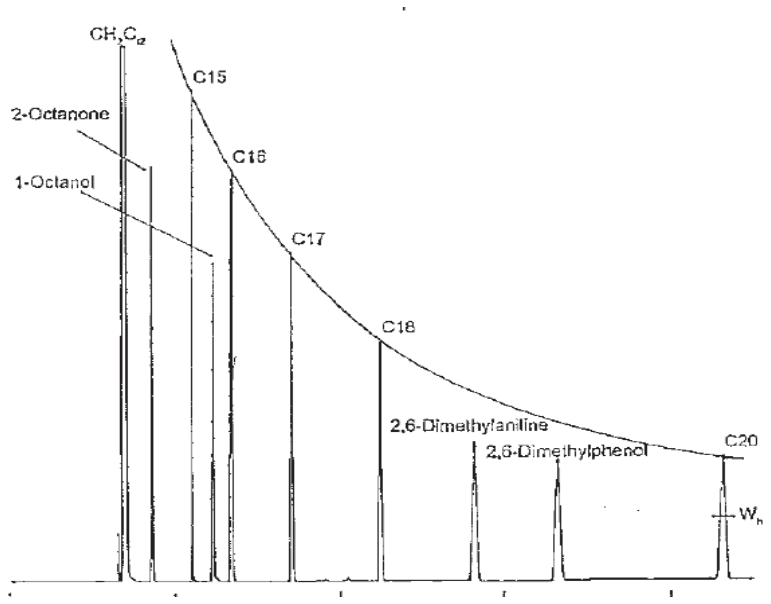
The following test is proposed for qualification of this type of column, using a mixture of nine compounds and an FID detector:

Chromatographic settings for 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

Chromatographic settings for 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.

Solutions:
Prepare a mixture of the nine compounds in solvent methylene chloride, with the following concentrations:
- 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-dimethylphenole – 0.5 mg/ml

Method:
After the system is equilibrated, inject the prepared mixture at least twice. Calculate the retention factor, selectivity for the adjacent peaks and plates per meter. Calculate the percentage of the peak height of n-alkanes by drawing a line connecting the peak maxima of these non-adsorbing peaks and calculate the % response for active compounds using the equation given above in the test for non-polar columns.



Criteria for characterisation and limits:

- The elution order is: 2-octanone; N-pentadecane; 1-octanol; N-hexadecane; N-heptadecane; N-octadecane; 2,6-dimethylaniline; 2,6-dimethylphenol; N-eicosane.
- Methane – this component is used as an indicator for the hold-up time (t_M), which is required to calculate the retention factor (k) and mean linear gas velocity in the column ($\bar{u}$ = column length in cm/gas hold-up time in cm). Tailing of the peak indicates dead volume in the system.
- 2,6-dimethylaniline - tailing or reduced peak height for this component indicates that the active sites are acidic.
- 2,6-dimethylphenol – tailing or reduced peak heights for this component indicates that the active sites are basic.
- α for 2,6-dimethylaniline/2,6-dimethylphenol represents the acid/base ratio.
- 1-octanol - tailing or reduced peak heights indicates silanol or hydrogen-bonding sites. This is a very sensitive probe for deactivation problems.
- 2-octanone - tailing or reduced peak heights indicate Lewis acid sites.
- The last eluting hydrocarbons (with k between 5 and 7) are used for the calculation of column efficiency.

Limits:

- A_s = 0.8 to 1.5
- The number of plates per meter for the peak with k between 5 and 7: the requirements are given in Table 1 (see Chapter 7 above) according to the internal diameter and polarity
- α between adjacent peaks ≥ 1.5
- % response for active component ≥ 70%.

ANNEX 2:

REQUIREMENTS FOR THE CONTENT OF A QUALIFICATION RECORD

Following the qualification, the OMCL should issue a qualification record including the following information:

- Title and identification of the record
- Page numbers
- Date of qualification
- Reference to the procedure for qualification
- Reference material or reagents used
- Results
- Chromatographic equipment used
- Conclusion (PASS/FAIL)
- Name and signature of the person who performed the qualification