Pharmacopeias: overview, uses and related activities

A guide towards a correct use

University of Pavia (Italy)

17th November 2023 Aula Magna – Collegio A. Volta

AN IMPORTANT GENERAL CHAPTER: PH. EUR. 2.2.46 AND HARMONIZED TEXT

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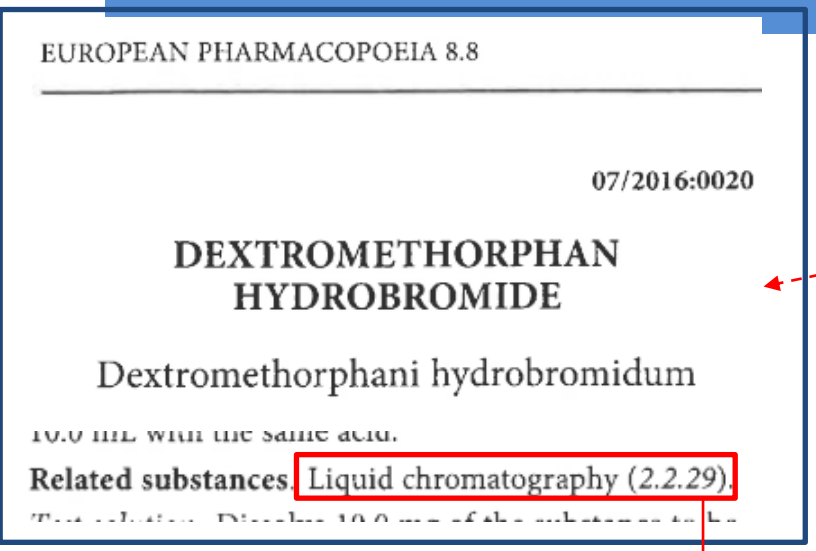
Ph. Eur 11:2.2.46 – USP <621> – Supplement I, JP XVIII 2.0 WHO 1.14.1 CHROMATOGRAPHY (draft)

Content (TLC, **HPLC**, GC, ~~SFC~~)

- **Definitions**
  - *Be able to calculate parameters in a paper chromatogram by using a pencil and a ruler*
- **System suitability**
  - *Small variations since Ph. Eur. 10: $0.8 \leq A_s \leq 1.8$*
- **Adjustment of chromatographic conditions**
  - *object of this presentation*
- **Quantitation**
- **Other considerations (new)**
  - *Tangential skimming*
  - *Correction of impurity relative response factor if* |rel. resp – 1|>0.2

Individual monographs and chapter 2.2.46



EUROPEAN PHARMACOPOEIA 8.8

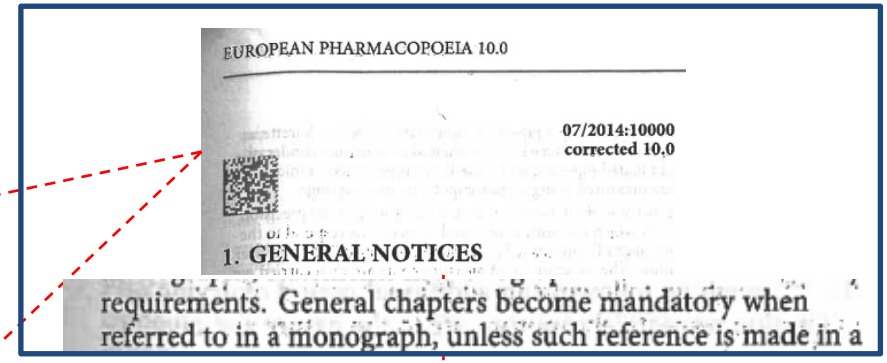
07/2016:0020

DEXTROMETHORPHAN HYDROBROMIDE

Dextromethorphani hydrobromidum

10.0 mL with the same acid.

Related substances. Liquid chromatography (*2.2.29*).

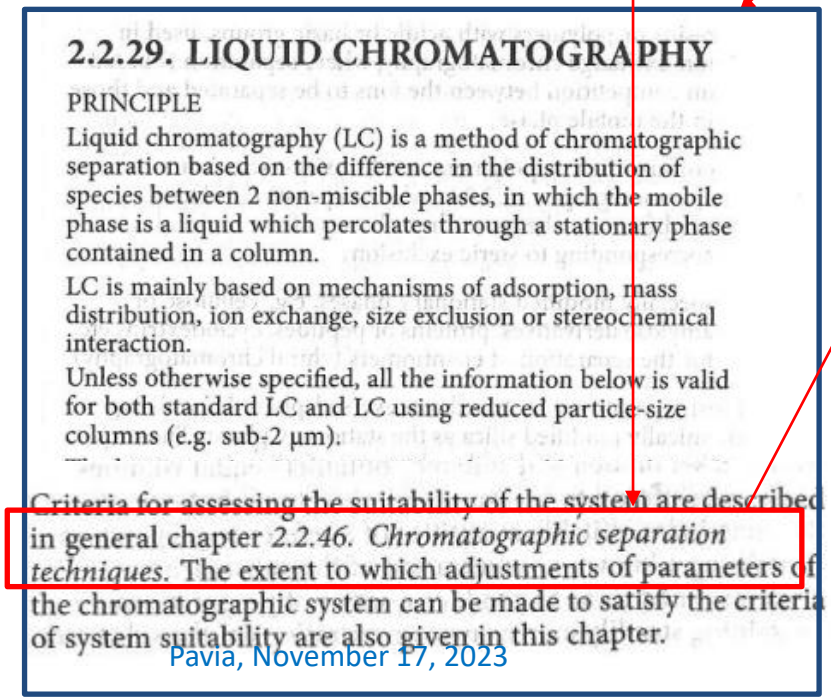


EUROPEAN PHARMACOPOEIA 10.0

07/2014:10000
corrected 10.0

1. GENERAL NOTICES

requirements. General chapters become mandatory when referred to in a monograph, unless such reference is made in a



2.2.29. LIQUID CHROMATOGRAPHY

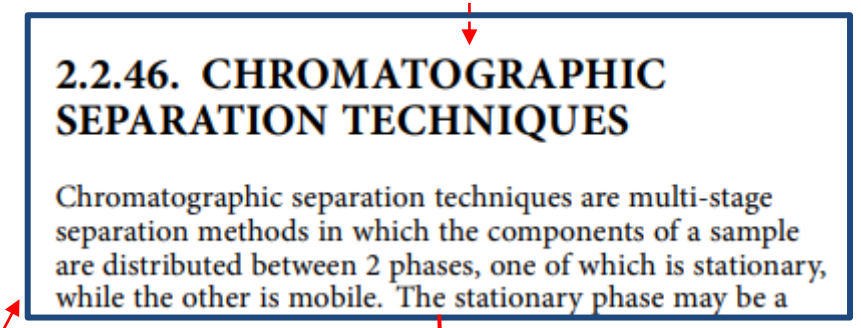
PRINCIPLE

Liquid chromatography (LC) is a method of chromatographic separation based on the difference in the distribution of species between 2 non-miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column.

LC is mainly based on mechanisms of adsorption, mass distribution, ion exchange, size exclusion or stereochemical interaction.

Unless otherwise specified, all the information below is valid for both standard LC and LC using reduced particle-size columns (e.g. sub-2 µm).

Criteria for assessing the suitability of the system are described in general chapter *2.2.46. Chromatographic separation techniques*. The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.



2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES

Chromatographic separation techniques are multi-stage separation methods in which the components of a sample are distributed between 2 phases, one of which is stationary, while the other is mobile. The stationary phase may be a

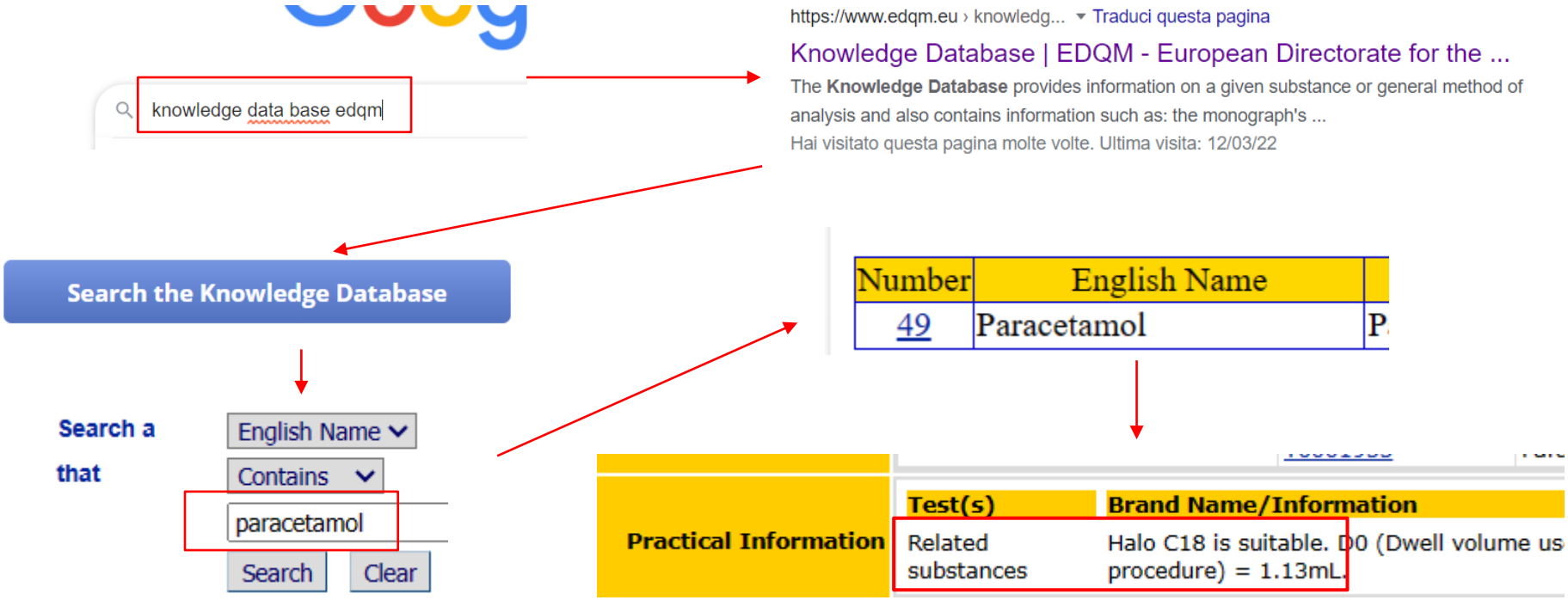
Definitions
System Suitability Test
Adjustment of chromatographic conditions
Quantitation
Other considerations

Reasons of the *Adjustment of chromatographic conditions* paragraph

- **Up to '90ies: all chromatographic parameters fixed**, problems:
  - small deviations from the system suitability test can be overcome by small adjustments of chromatographic conditions,
  - *"Nonetheless, since the stationary phases are described in a general way, with differences in chromatographic behaviour, some adjustments of the chromatographic conditions may be necessary to achieve the prescribed system suitability requirements…"*
- Ph. Eur. 10: the system suitability test is the only qualification criterion.
- Ph. Eur. 10 : very limited adjustments to gradient conditions.

"Chemistry" of the column (EDQM)

- Pharmacopoeias never give commercial indications on a reagent (or column) trade mark.
- The exact column used in development of Ph. Eur. procedures can be found in the Knowledge Data Base of the EDQM site (free access).



Introduction: Ph. Eur. 11 vs. Ph. Eur. 10

Ph. Eur. 10

GB: since Ph. Eur. 6: one point corrective action

The extent to which the various parameters of a chromatographic test may be **adjusted to satisfy the system suitability criteria** without fundamentally modifying the method are listed below

Changes other than those indicated require revalidation of the method. The chromato-graphic conditions described have been validated during the elaboration of the monograph.

Ph. Eur. 11

The chromatographic conditions described have been validated during the elaboration of the monograph.
The extent to which the various parameters of a chromatographic test may be **adjusted without fundamentally modifying the pharmacopeial analytical procedures** are listed below. Changes other than those indicated require validation of the procedure.

If adjustments are made to a pharmacopeial procedure, additional verification tests may be required. To verify the suitability of the adjusted pharmacopoeial procedure, assess the relevant analytical performance characteristics potentially affected by the change.

→ *Risk assessment - Lifecycle of the anal proc.*

Multiple adjustment

Multiple adjustments can have a cumulative effect on the performance of the system and are to be properly evaluated by the users. This is particularly important in cases where the separation pattern is described as a profile. In those cases, a risk assessment has to be carried out.

but in Ph. Eur. 11,

(isocratic conditions, after adjustment of column & particles geometry:

When a change is made from ≥ 3 µm to < 3 µm particles in isocratic elution, an additional increase in linear velocity (by adjusting the flow rate) may be justified, provided that the column performance does not drop by more than 20 per cent. Further adjustments in analytical procedure conditions (mobile phase, temperature, pH, etc.) may be required, within the permitted ranges described under System Suitability and Adjustment of chromatographic conditions in this chapter.

more consideration of "technically inherent justifications"
MODR concept not included

New: superficially porous particles

- *Stationary phase*: no change of the identity of the substituent (e.g. no replacement of C18 by C8);
- the other physico-chemical characteristics of the stationary phase (i.e. chromatographic support, surface modification and extent of chemical modification) must be similar ;
- a change from totally porous particle (TPP) columns to superficially porous particle (SPP) columns is allowed provided the above-mentioned requirements are met.

- An example (paracetamol):
  - *end-capped solid core octadecylsilyl silica gel for chromatography R*

Column dimensions (particlesize, length):

- the particle size and/or length of the column may be modified provided that the ratio of the column length (*L*) to the particle size (*dp*) remains constant or in the range − 25 per cent to + 50 per cent of the prescribed *L/dp* ratio. For the application of particle-size adjustment from totally porous to superficially porous particles, other combinations of *L* and *dp* can be used provided that the plate number (*N*) is within − 25 per cent to+ 50 per cent relative to the prescribed column.
- These changes are acceptable provided the system suitability requirements are fulfilled and the selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent. (GB: also for GC)

GB: constant L/dp ratio → constant plate number (Knox equation, 1977): in column transfer, at constant reduced linear velocity (ν) the reduced plate height (h) is constant:

$$h = \frac{H}{d_p} \qquad \nu = u \cdot \frac{d_p}{D_m}$$

Adjustments when colum geometry (dc, L) and/or particle diameter (dp) are changed

- Flow rate:

$$F_2 = F_1 \times \frac{dc_2^{\,2} \times dp_1}{dc_1^{\,2} \times dp_2}$$

(derived from the Knox equation)

- Injection volume:

$$V_{inj2} = V_{inj1} \times \frac{L_2 \times dc_2^{\,2}}{L_1 \times dc_1^{\,2}}$$

(to take into account plate volume)

- When the injection volume is decreased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined.
- An increase is permitted provided that, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.

Suggestion for training: the use of simulators

HPLC simulator of the Université de Genève (an Excel file with macro, 7 example mixtures):

https://ispso.unige.ch/labs/fanal/practical_hplc_simulator:en

Adjustments of isocratic conditions

- Mobile phase
  - composition,
  - pH of the aqeous component
  - Concentration of the salt in the buffer component
  - Flow rate

Small changes from Ph. Eur 10

Inverted order vs. geometric changes.

GRADIENT ELUTION

- Fewer adjustments allowed:
  - flow rate not listed as adjustable (rational: gradient volume changes and retention order can change) unless granulometry change and/or column geometry change;
  - mobile phase/gradient adjustments:
    - the principal peak(s) elute(s) within ± 15 per cent of the indicated retention time(s) obtained with the original conditions; this requirement does not apply when the column dimensions are changed;
    - the composition of the mobile phase and the gradient are such that the first peaks are sufficiently retained and the last peaks are eluted. (*Ph. Eur. 10: the final composition of the mobile phase is not weaker in elution power*.)
  - Dwell volume to be adapted to chromatograph (dwell volume of the chromatogram used for monograph elaboration on the Knowledge Database).

GRADIENT: adjustment in column/particle geometry

- In case of change of column/particle geometry
  - adjust flow rate,
    (see isocratic elution)
  - adjust injection volume,
    (see isocratic elution)

  - adjust the gradient time to keep the same gradient volume:

$$t_{G2} = t_{G1} \times \frac{F_1}{F_2} \times \frac{L_2 \times dc_2^{\,2}}{L_1 \times dc_1^{\,2}}$$

Qualification (validation) of adjustments in Ph. Eur. 11

- Compliance with the system suitability criteria is required to verify that conditions for satisfactory performance of the test or assay are achieved.

- These changes are acceptable provided the system suitability requirements are fulfilled and the selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.

- When the injection volume is decreased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined
- An increase is permitted provided that, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.

✓ more freedom, possibility to adopt technology advancements,
✓ more responsability (more validation).
✓ Ph. Eur. 5.26 a useful guide (risk analysis + experimental work)?

Not new, but important

For some parameters, the adjustments are explicitly defined in the monograph to ensure the system suitability.

The PAR concept (Permitted Acceptable Range, guideline ICH Q14)

An example (methotrexate – related substances, gradient method):

System Suitability

....

if the resolution between impurity D and methotrexate does not comply, increase the flow rate to meet the requirement.

A comment on a table (isocratic conditions adjustment)

Table 2.2.46.-2. – *Example of adjustments for liquid chromatography - gradient elution*

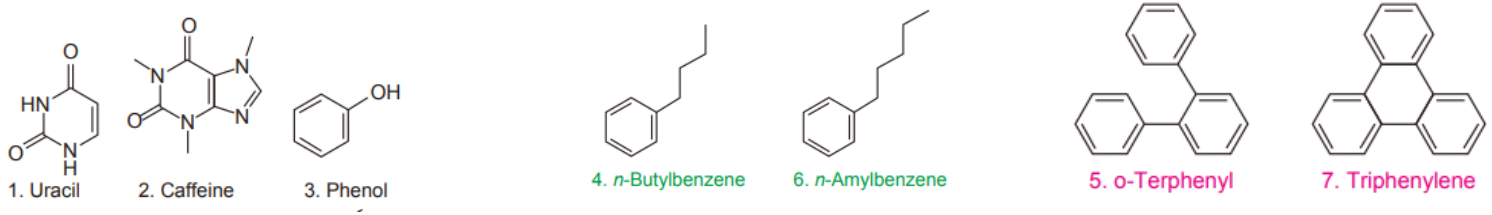
Variable	Original conditions	Adjusted conditions	Comment
Column length (L) in mm	150	100	User's choice
Column diameter (dc) in mm	4.6	2.1	User's choice
Particle size (dp) in µm	5	3	User's choice
L/dp	30.0	33.3	(1)
Flow rate (F) in mL/min	2.0	0.7	(2)
Gradient adjustment factor (t_{G2}/t_{G1})		0.4	(3)
Gradient conditions			
B (per cent)	*Time (min)*	*Time (min)*	
30	0	0	
30	3	$(3 \times 0.4) = 1.2$	
70	13	$[1.2 + (10 \times 0.4)] = 5.2$	
30	16	$[5.2 + (3 \times 0.4)] = 6.4$	

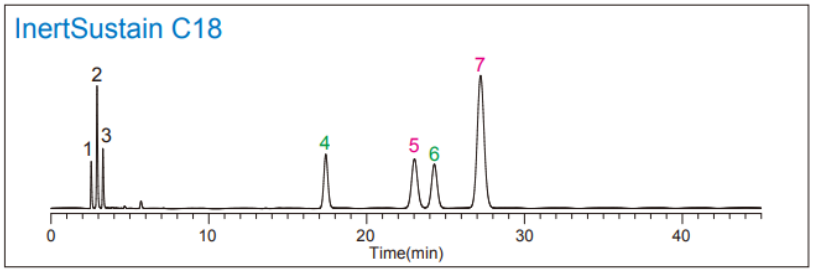
it work if* the exact chemistry** is the same after adjustment

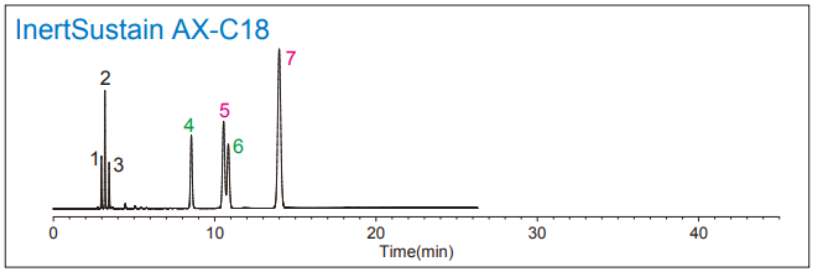
* not necessarily only if
** the particle brand

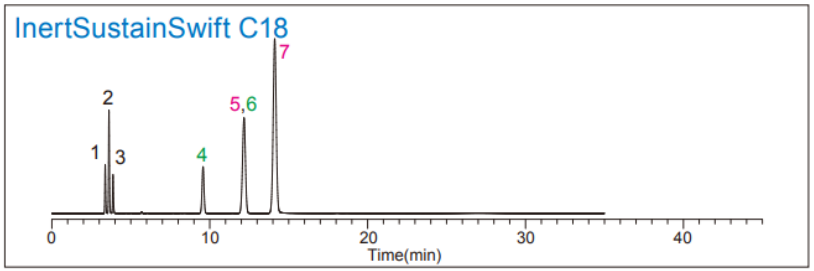
Go back to the chemistry of the stationary phase
or
column equivalency

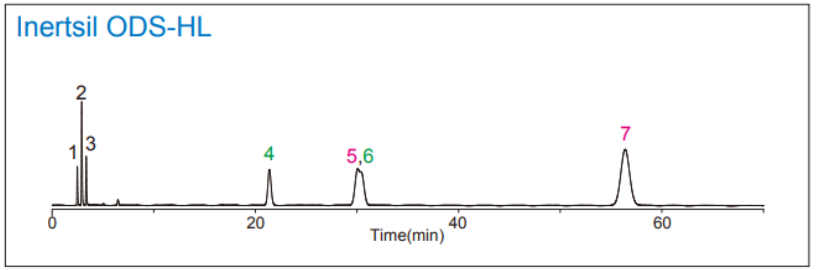
«It's a simple C18!»

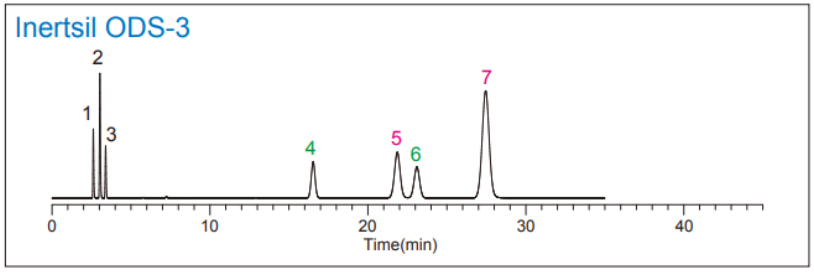


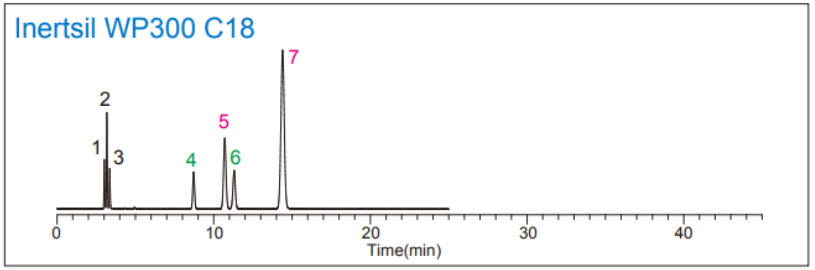








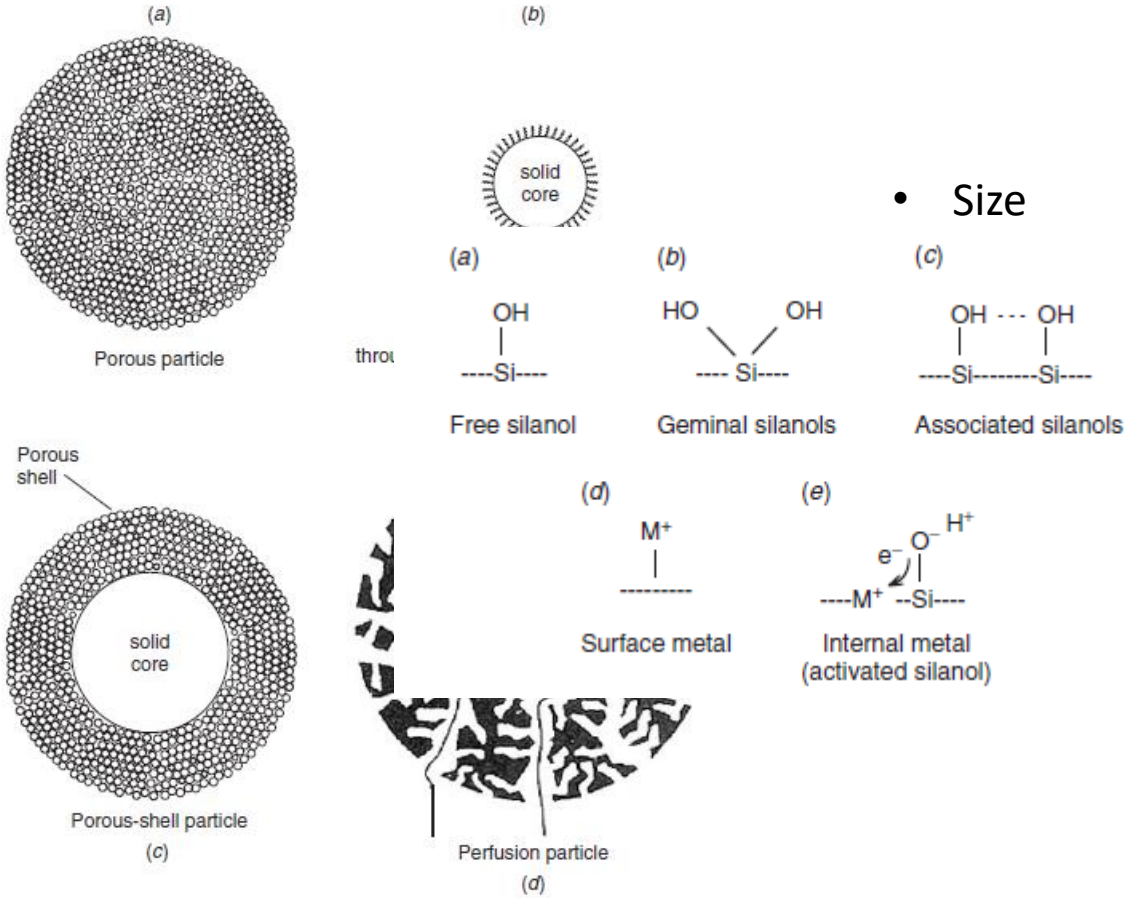




The properties of the column

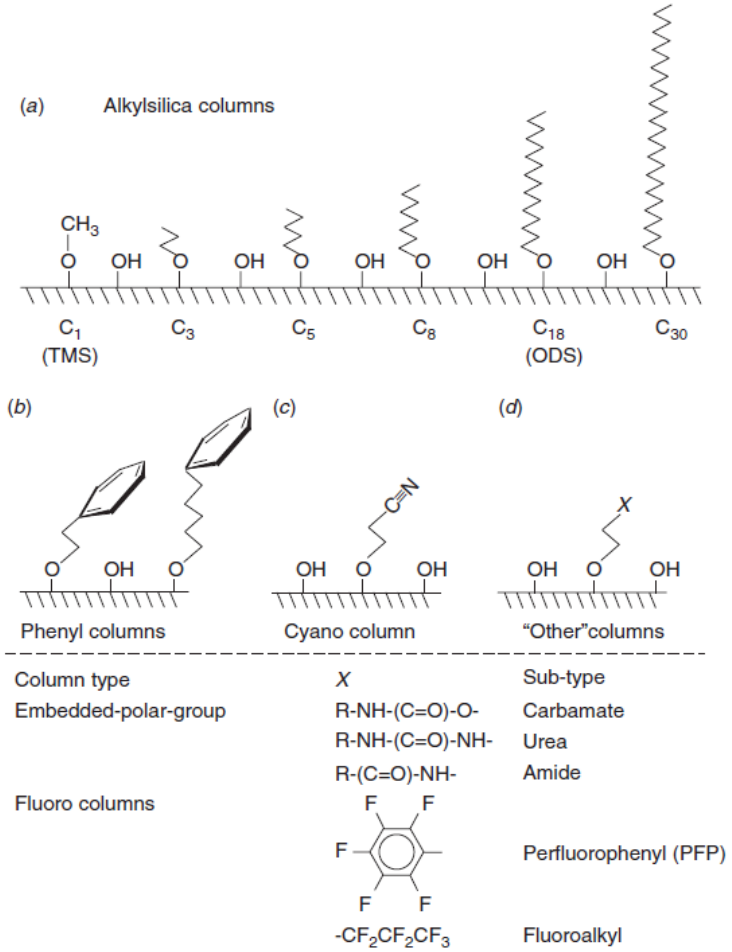
- Packing materials and related features
- Functionalisation and related features
- Packing Technology and quality

Packing materials and related features



- Size

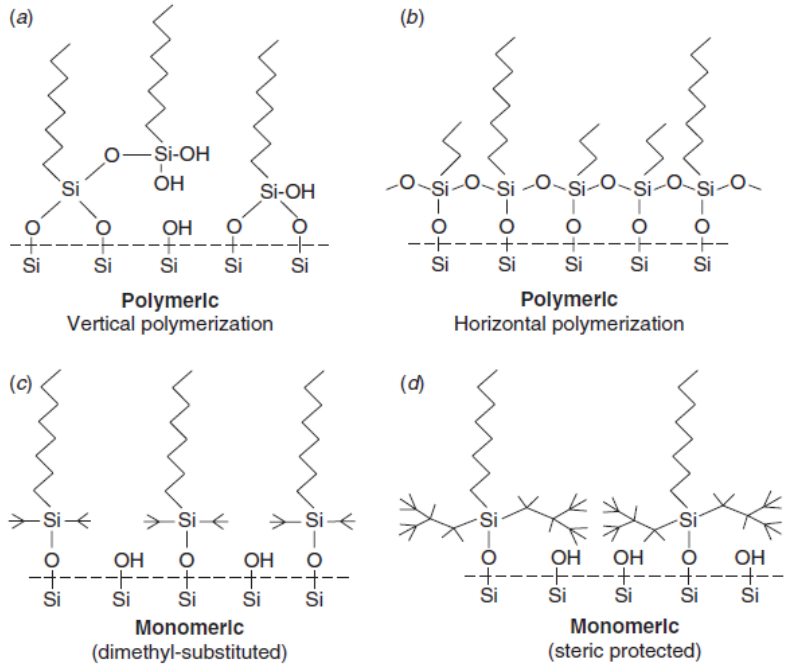
Functionalisation and related features



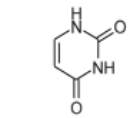
Column type	X	Sub-type
Embedded-polar-group	R-NH-(C=O)-O-	Carbamate
	R-NH-(C=O)-NH-	Urea
	R-(C=O)-NH-	Amide
Fluoro columns		Perfluorophenyl (PFP)
	$-CF_2CF_2CF_3$	Fluoroalkyl

Functionalisation and related features

- chemical reaction of functionalisation
- How many alkyl chains (ligand density/ % Carbon)?
- Type of endcapping
- Endcapping technology



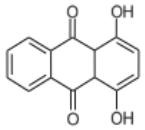
Come si notano queste differenze?



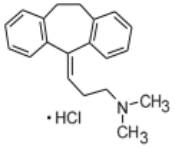
uracil - void volume marker

toluene - hydrophobic retention, methylene selectivity

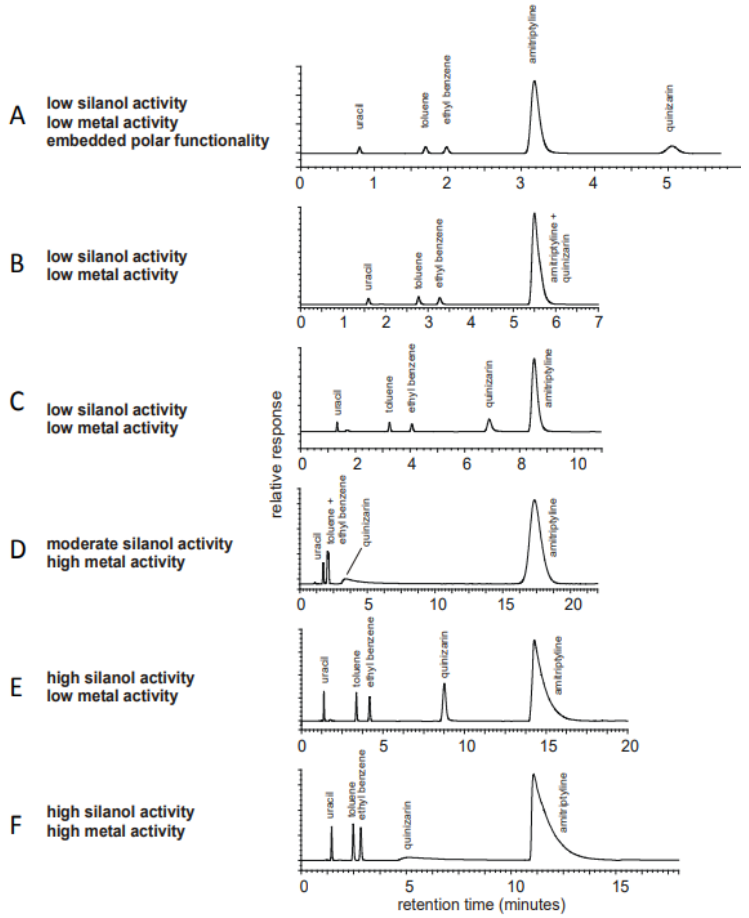
ethyl benzene - hydrophobic retention, methylene selectivity



quinizarin - activity towards chelating reagents

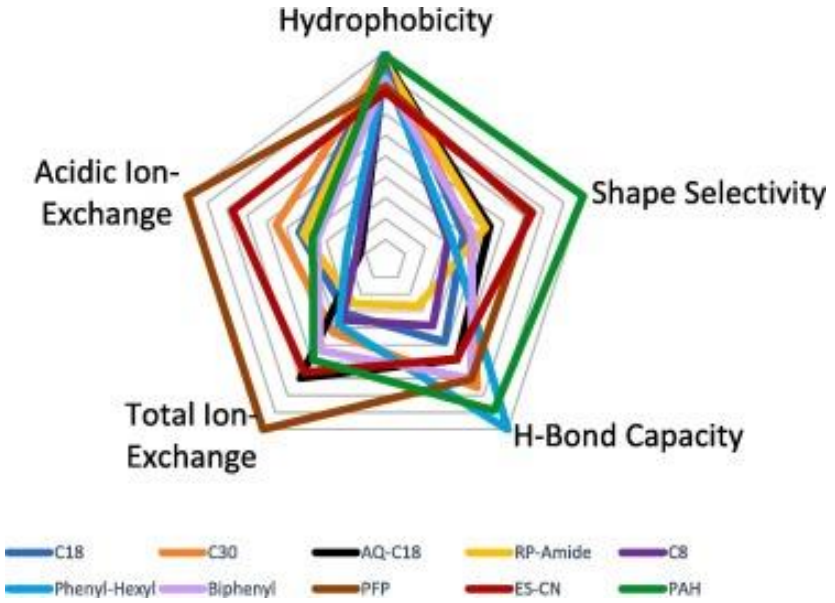


amitriptyline hydrochloride - activity towards bases



I Tools

- Tanaka test*
- NIST**
- PQRI***



* McHale, Conner, et al. "A Simple Approach for Reversed Phase Column Comparisons via the Tanaka Test." *Microchemical Journal*, vol. 162, Mar. 2021, p. 105793, https://doi.org/10.1016/j.microc.2020.105793

"Column Selection for Reversed-Phase HPLC." LCGC North America, vol. 31, no. 3, 1 Mar. 2013, pp. 262–262, www.chromatographyonline.com/view/column-selection-reversed-phase-hplc

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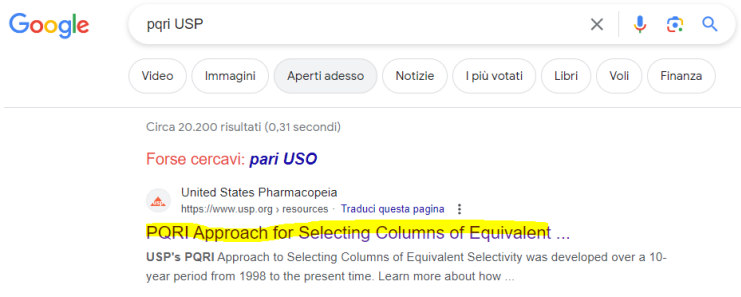
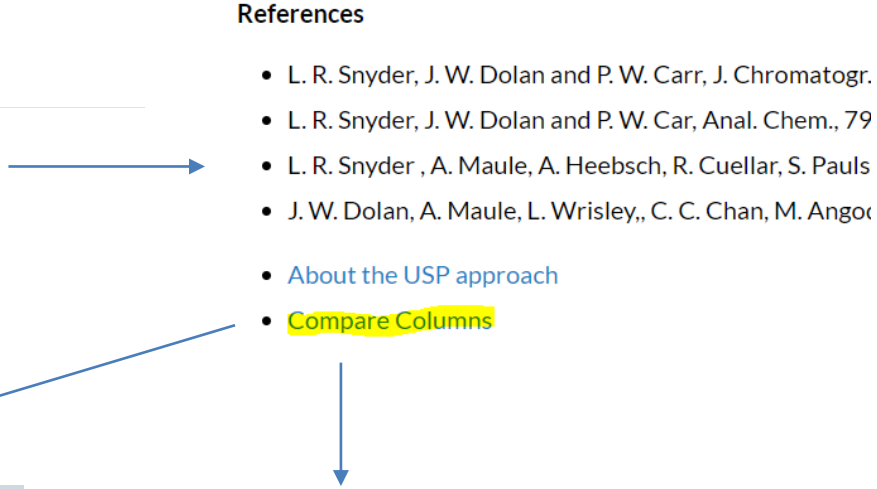
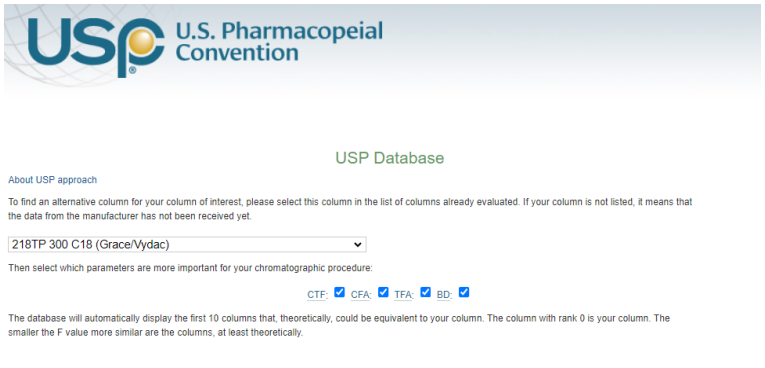
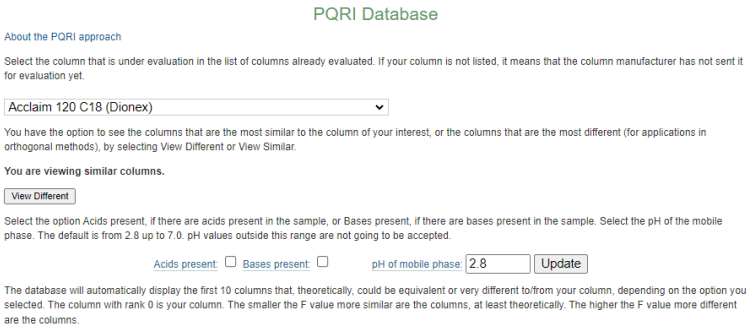
USP Pharmacopoeial forum 31(2)

USP Tools


Google
pqri USP
Video
Immagini
Aperti adesso
Notizie
I più votati
Libri
Voli
Finanza
Circa 20.200 risultati (0,31 secondi)
Forse cercavi: pari USO
United States Pharmacopeia
https://www.usp.org › resources · Traduci questa pagina
PQRI Approach for Selecting Columns of Equivalent ...
USP's PQRI Approach to Selecting Columns of Equivalent Selectivity was developed over a 10-year period from 1998 to the present time. Learn more about how ...


References
L. R. Snyder, J. W. Dolan and P. W. Carr, J. Chromatogr.
L. R. Snyder, J. W. Dolan and P. W. Car, Anal. Chem., 79
L. R. Snyder , A. Maule, A. Heebsch, R. Cuellar, S. Pauls
J. W. Dolan, A. Maule, L. Wrisley,, C. C. Chan, M. Angoc
About the USP approach
Compare Columns


U.S. Pharmacopeial Convention
USP Database
About USP approach
To find an alternative column for your column of interest, please select this column in the list of columns already evaluated. If your column is not listed, it means that the data from the manufacturer has not been received yet.
218TP 300 C18 (Grace/Vydac)
Then select which parameters are more important for your chromatographic procedure:
CTF: CFA: TFA: BD:
The database will automatically display the first 10 columns that, theoretically, could be equivalent to your column. The column with rank 0 is your column. The smaller the F value more similar are the columns, at least theoretically.


PQRI Database
About the PQRI approach
Select the column that is under evaluation in the list of columns already evaluated. If your column is not listed, it means that the column manufacturer has not sent it for evaluation yet.
Acclaim 120 C18 (Dionex)
You have the option to see the columns that are the most similar to the column of your interest, or the columns that are the most different (for applications in orthogonal methods), by selecting View Different or View Similar.
You are viewing similar columns.
View Different
Select the option Acids present, if there are acids present in the sample, or Bases present, if there are bases present in the sample. Select the pH of the mobile phase. The default is from 2.8 up to 7.0. pH values outside this range are not going to be accepted.
Acids present: Bases present: pH of mobile phase: 2.8 Update
The database will automatically display the first 10 columns that, theoretically, could be equivalent or very different to/from your column, depending on the option you selected. The column with rank 0 is your column. The smaller the F value more similar are the columns, at least theoretically. The higher the F value more different are the columns.

The use of USP Tools

PQRI Database

About the PQRI approach

Select the column that is under evaluation in the list of columns already evaluated. If your column is not listed, it means that the column manufacturer has not sent it for evaluation yet.

YMC-Triart C18 (YMC)

You have the option to see the columns that are the most similar to the column of your interest, or the columns that are the most different (for applications in orthogonal methods), by selecting View Different or View Similar.

You are viewing similar columns.

View Different

Select the option Acids present, if there are acids present in the sample, or Bases present, if there are bases present in the sample. Select the pH of the mobile phase. The default is from 2.8 up to 7.0. pH values outside this range are not going to be accepted.

Acids present: ☐ Bases present: ☐ pH of mobile phase: 2.8 Update

The database will automatically display the first 10 columns that, theoretically, could be equivalent or very different to/from your column, depending on the option you selected. The column with rank 0 is your column. The smaller the F value more similar are the columns, at least theoretically. The higher the F value more different are the columns.

Rank	F	Column	H	S	A	B	C(2.8)	C(7.0)	Type	USP Designation	Manufacturer
0	0	YMC-Triart C18	0.929	-0.02	-0.19	-0.033	-0.023	-0.139	B	L1	YMC
1	0.56	Sepax HP-C18(2)	0.959	-0.024	-0.187	-0.007	-0.134	0.055	B	L1	Sepax Technologies
2	0.57	Fortis C18	0.96	-0.023	-0.18	-0.009	-0.167	0.111	B	L1	Fortis Technologies
3	0.58	HSS T3	0.949	-0.021	-0.173	-0.002	0.031	0.18	B	L1	Waters
4	0.72	Acclaim300 C18	0.957	-0.018	-0.17	0.019	0.261	0.222	B	L1	Dionex
5	0.81	Sunniest RP-AQUA	0.958	-0.024	-0.21	-0.008	0.142	0.098	EP	L60	Chromanik
6	0.84	Epic C18	0.95	-0.027	-0.203	-0.007	-0.131	-0.041	B	L1	ES Industries
7	0.97	Inspire C8	0.889	-0.025	-0.212	-0.004	-0.193	-0.014	B	L7	Dikma Technologies
8	1.11	Atlantis dC18	0.917	-0.031	-0.193	0.001	0.036	0.087	B	L1	Waters
9	1.11	Athena C18-WP	0.953	-0.03	-0.203	-0.003	-0.052	0.066	B	L1	CNW Technologies
10	1.11	Xtimate C8	0.855	-0.014	-0.185	0.008	0.013	0.173	B	L7	Welch

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About the PQRI approach

The use of USP Tools

PQRI Database

About the PQRI approach

Select the column that is under evaluation in the list of columns already evaluated. If your column is not listed, it means that the column manufacturer has not sent it for evaluation yet.

YMC-Triart C18 (YMC)

You have the option to see the columns that are the most similar to the column of interest, or the columns that are the most different (for applications in orthogonal methods), by selecting View Different or View Similar.

You are viewing similar columns.

View Different

Select the option Acids present, if there are acids present in the sample, or Bases present, if there are bases present in the sample. Select the pH of the mobile phase. The default is from 2.8 up to 7.0. pH values outside this range are not going to be accepted.

Acids present: ☐ Bases present: ☑ pH of mobile phase: 2.8 Update

The database will automatically display the first 10 columns that, theoretically, could be equivalent or very different to/from your column, depending on the option you selected. The column with rank 0 is your column. The smaller the F value more similar are the columns, at least theoretically. The higher the F value more different are the columns.

Rank	F	Column	H	S	A	B	C(2.8)	C(7.0)	Type	USP Designation	Manufacturer
0	0	YMC-Triart C18	0.929	-0.02	-0.19	-0.033	-0.023	-0.139	B	L1	YMC
1	1.35	Inertsil ODS-4	0.911	-0.026	-0.226	-0.03	-0.029	-0.143	B	L1	GL Sciences
2	1.7	Targa C8	0.821	-0.023	-0.221	0.004	-0.027	0.174	B	L7	Higgins Analytical
3	1.85	Luna Omega C18	0.976	-0.003	-0.187	-0.007	-0.018	0.005	B	L1	Phenomenex
4	2.03	Develosil ODS-MG-5	0.963	-0.036	-0.165	-0.003	-0.012	0.051	B	L1	Nomura
5	2.51	Ace 5 C18-PFP	0.899	-0.021	-0.246	-0.08	-0.001	-0.995	B	L1	ACT
6	2.54	Inertsil ODS-SP	0.858	-0.027	-0.221	-0.023	-0.048	-0.073	B	L1	GL Sciences
7	2.65	Athena C18-WP	0.953	-0.03	-0.203	-0.003	-0.052	0.066	B	L1	CNW Technologies
8	2.84	Cosmicsil Aura ODS	0.948	-0.04	-0.185	0.009	-0.047	0.089	B	L1	Genius Technologies
9	2.95	Orosil C18	0.981	-0.032	-0.137	0.002	-0.048	0.155	B	L1	Orochem Technologies
10	3.04	Aeris WIDEPORE XB-C8	0.788	-0.038	-0.169	0.073	-0.042	0.518	B	L7	Phenomenex

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Conclusions

- ✓ More freedom, possibility to adopt technology advancements,
- ✓ more responsability (more validation).
- ✓ Ph. Eur. 5.26 a useful guide (risk analysis + experimental work)?
- ✓ A simple but solid base on chromatographic theory is advisable to correctly apply the chapters.
- ✓ USP seems to encourage the use of good science in column changes.

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