Equilibration – *As much as necessary, as little as possible*

During equilibration and rinsing a column, the following question often arises: “For how long do I have to flush the column?” If the (re)equilibration time is too short, there is a risk of non-reproducible results (e.g. retention time shift). If in doubt a longer duration of flushing should be favoured. But if the rinse time is too long, time and, especially for preparative methods, solvents are wasted.

Practical example

A YMC-Triart C8 column (TO12S03-1546WT, column volume 2.5 mL) was rinsed with 50 mL of acetonitrile before each run. A plant extract was injected under four different conditions:

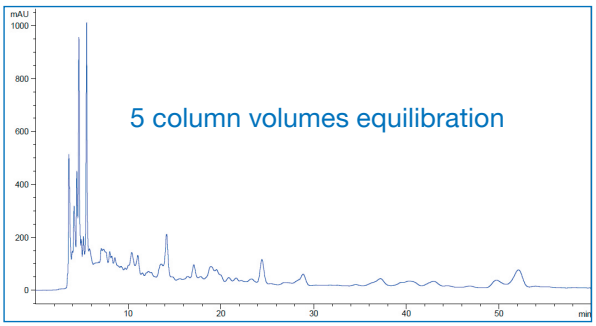
- without Equilibration
- Equilibration with 1 column volume
- Equilibration with 5 column volumes
- Equilibration with 10 column volumes

Equilibration and isocratic separation was performed with eluent: water/acetonitrile (80/20).
The dwell volume of the system was 130 µL.

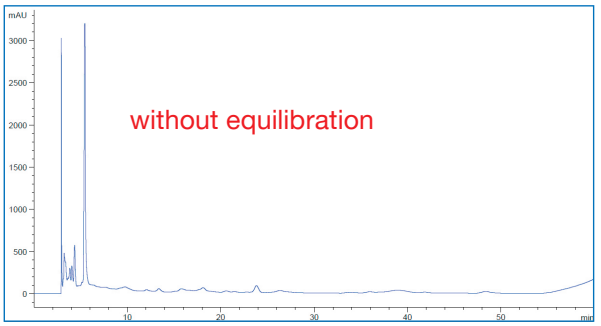
Result

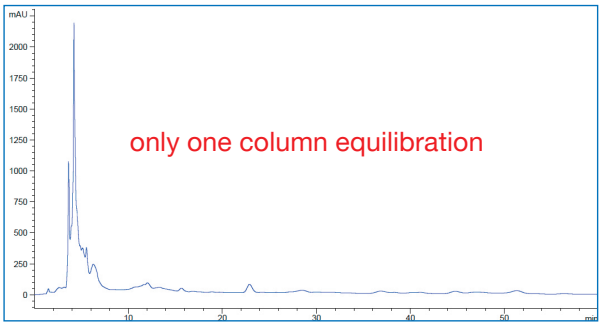
It is obvious, without sufficient equilibration the retention time shifts to shorter times. When using 5 or 10 column volumes, the results of this application are reproducible. Equilibration with 5 column volumes is most efficient.

1. The application:

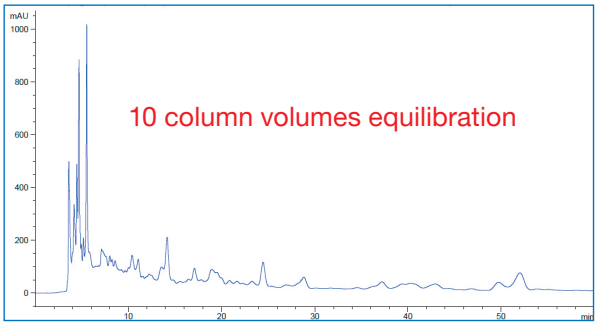


2. Operating without or with insufficient equilibration - retention time shifts occur:





3. Here the equilibration is sufficient, but longer than necessary:



But how can I estimate the optimum equilibration time?

With the help of the column volume!

Calculation of column volume:

Geometric column volume [mL] **= Length** [cm] **× (Radius** [cm]**)² × π**

Example:

Column: YMC-Triart C18; TA12S03-2546WT
Column dimension: 250 mm length; 4.6 mm inner diameter
Column volume = 25 cm × (0.23 cm)² × 3.14
= 4.2 mL

What else needs to be considered?

Dwell volume

- Volume until the solvent reaches the column
- dependent on the system*

How can I shorten the equilibration time?

Increase the flow rate during equilibration – the faster the necessary volume is reached.
The pressure limitation of hardware and stationary phase need to be considered*.

Overview of geometric column volumes [mL] **for selected dimensions:**

L [mm] / Ø [mm]	50	75	100	150	250	300
2.0	0.2	0.2	0.3	0.5	0.8	0.9
3.0	0.4	0.5	0.7	1.1	1.8	2.1
4.6	0.8	1.2	1.7	2.5	4.2	5.0
6.0	1.4	2.1	2.8	4.2	7.1	8.5
8.0	2.5	3.8	5.0	7.5	12.6	15.1
10.0	3.9	5.9	7.9	11.8	19.6	23.6
20.0	15.7	23.6	31.4	47.1	78.5	94.2
30.0	35.3	53.0	70.7	106.0	176.7	212.1
50.0	98.2	147.3	196.3	294.5	490.9	589.0

* Ask your supplier

When working with similar eluents (e.g. 10% ACN to 90% ACN) 10 column volumes are normally considered sufficient. After gradient elution, the equilibration can be shortened by considering the difference in percentage of eluent B. For example, if eluent B is increased from 20% to 60% the calculation is as follows: 10 column volumes × 0.4.

If the eluents are very different (e.g. changing from methanol to acetonitrile, addition of ion pairing reagents, etc.) or if normal phase conditions are used a minimum 20–30 column volumes are necessary – this would also be the same for cleaning and regeneration of columns. However, if no stable baseline or stable back pressure is reached, the rinsing time needs to be increased.

Conclusion

The geometric column volume is a very useful tool for calculation of the required eluent volume for flushing and equilibrating in HPLC – an important pre-requisite for reproducible and valid results.