Equilibration of HPLC columns

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"How long should I leave my HPLC column to equilibrate?" This is a question commonly asked by HPLC analysts, particularly those relatively new to the technique. The range of currently available column dimensions means that a 'one size fits all' approach is not appropriate. The amount of mobile phase which should be flushed through a column before it is ready to use is usually expressed in terms of the column volume, the amount of mobile phase required to fill the column.

When a column has just been installed on a reversed phase HPLC system then it will typically require between 10 and 20 column volumes before it is fully equilibrated and ready to use. However, some applications are likely to require additional column volumes. Examples are methods which include ion-pairing reagents and chiral HPLC methods. In these cases suitable equilibration should be investigated and documented for future use. A starting point for investigation may be approximately 30 column volumes. When re-equilibrating after a gradient injection has been run (prior to the next injection) 10 column volumes is recommended.

Although we refer to the column volume, the volume that we are interested in is more correctly called the void volume, V_m. This is the volume of the HPLC column that is not taken up by the stationary phase. This is typically approximately 70% of the total column volume. There are two methods that you can use to calculate the void volume:

Method 1

Use the dimensions of the column and the formula for the volume of a cylinder:

Volume of a cylinder, V:

$$V = \pi \times r^2 \times L$$

The dimensions of interest on a HPLC column are shown in **Figure 1**:

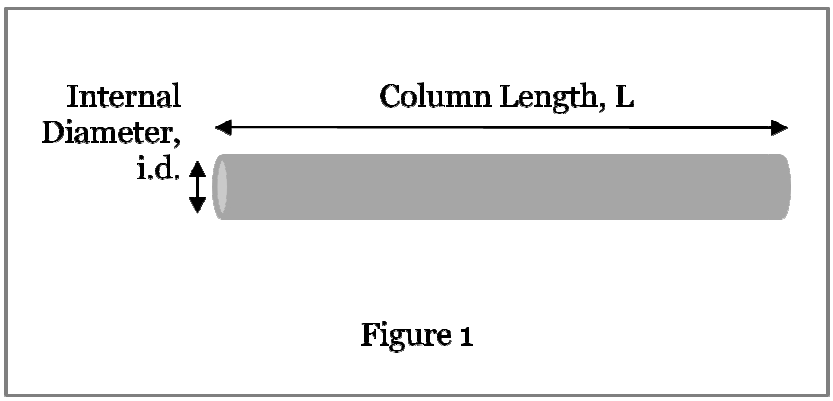

Figure 1

The result of the calculation above is the total column volume. To convert this value to the void volume we multiply by 70%, therefore the formula becomes:

Void volume, Vm:

$$V_m = 0.7 \times \pi \times r^2 \times L$$

The radius, r, is equal to the internal diameter divided by two:

Void volume, Vm:

$$V_m = 0.7 \times \pi \times \left(\frac{i.d.}{2}\right)^2 \times L$$

Example

A HPLC column of internal diameter 4.6mm and length 10cm:

$$V_m = 0.7 \times \pi \times \left(\frac{0.46}{2}\right)^2 \times 10$$
$$V_m = 1.16cm^3 = 1.16mL$$

Note: express both i.d. and length in cm

To calculate the required equilibration time simply multiply the calculated void volume (in mL) by the number of required column volumes, e.g. 10, then divide by the flow rate (in mL/min)to determine the total time required.

You can use the convenient MTS calculator for these calculations (at http://www.mournetrainingservices.co.uk/HPLC_calculator.xls).

Method 2

Inject an unretained solute to obtain t_0, the column dead time (minutes). Then multiply this by the flow rate to obtain the void volume:

Void volume, Vm:

$$V_m = F \times t_0$$

Where F is the flow rate expressed in mL/min

Example

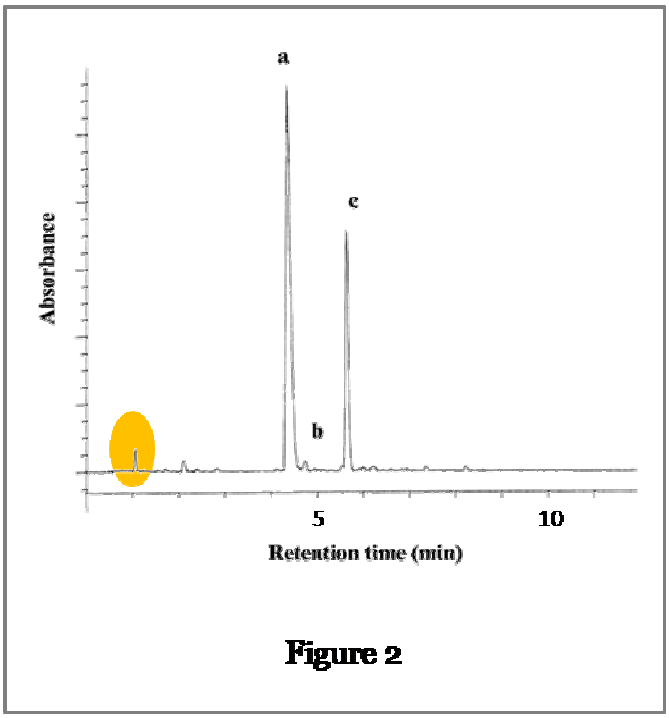

Figure 2

The flow rate for the method is 1.0 mL/min. The column dead time,t_0, is obtained from the chromatogram in **Figure 2** and is equal to 1.05 minutes.

Therefore V_m = 1.0 x 1.05 = 1.05mL

To calculate the required equilibration time from the void volume we divide by the flow rate. It can be seen that the above calculation involves multiplication by the flow rate. Therefore the equilibration time is simply the column dead time multiplied by the required number of column volumes, i.e. 1.05 x 10 = 10.5 minutes. The intention of providing the full calculation was to compare methods 1 and 2 since the void volume was calculated in each approach.

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