

## Determine pore volume of an HPLC column using HPLC injections – How To

**The interior of an HPLC column is contains tightly packed spherical particles. There are two types of empty space in the column, i.e. volume not occupied by these particles.**

The first type is the interstitial space between adjacent particles. This actually comprises a small percentage of the total void volume. The majority of the empty space is the pores of the particles (*the second type*). These pores are what gives silica its very high surface area, an essential feature for obtaining high loading **capacity** (*more active sites*).

The pore volume itself is not readily calculated by HPLC methodologies directly, but it can be obtained by difference. To do so, you would inject

- 1) a small, non-retained solute that *can enter the pores*
- 2) a large, un-retained solute that is *excluded from the pores*.

Measure the retention times of each one and take the difference (*small solute minus large solute*). This gives you the retention time of a hypothetical solute that would enter only the pores and not the interstitial space between the particles (*recall we are interested in the Pore Volume*). Because no such solute exists, you have to determine this retention time indirectly from our subtraction calculation.

Now to get the pore volume, you simply multiply this value by the flow rate. The equation can be summarized as follows:

$$\text{Pore Volume} = (t_{R \text{ small solute}} - t_{R \text{ large solute}}) * \text{Flow Rate}$$

*Be sure to use the same time units in retention time and flow rate so that the time units cancel and you are left with volume (mL).*

