

**Q). How to calculate time for injecting 5ml of sample, with 20 ml flow rate**

**(in method creation)?**

A). Basic formula for time calculation is

$$\frac{\text{Total Time}}{\text{Set Flow}} * \text{Injection Volume}$$

Here, Total Time = 100sec (fixed).

Set Flow Rate = 20ml

Injection Volume = 5ml

i.e.,

$$\frac{100}{20} * 5 = 25 \text{seconds.}$$

In case of 2ml injection, time calculation is

i.e.,

$$\frac{100}{20} * 2 = 10 \text{seconds.}$$

In case of 2ml injection, with 10ml injection flow, then time calculation is

i.e.,

$$\frac{100}{10} * 2 = 20 \text{seconds.}$$

**Example Method for injecting 2ml sample**

Time (Min)	Injection Pump A (%)	Solvent B (%)	Solvent C (%)	Total Flow
Initial	0.0	100.00	0.0	50.00ml
2.80	0.0	100.00	0.0	50.00 ml
2.81	100.00	0.0	0.0	20.00ml
3.00	100.00	0.0	0.0	20.00 ml
3.01	0.0	100.00	0.0	50.00 ml
5.00	0.0	100.00	0.0	50.00 ml

## DAC AND COLLAPSED COLUMN CALCULATIONS

**Q). what is the formula for finding total volume of the column**

A). Column Volume (CV) =  $\pi R^2 L$

Here,  $\pi = 3.14$ .

R = Radius of the column in millimetres.

L = Length of the column in millimetres.

**Q). what is the total volume for 50mm ID column with packing bed length of 350mm?**

A). Column Volume (CV) =  $\pi R^2(B. L)$

**Note:** Measurements in millimetres, must have to consider in centimetres.

Here, Column ID in centimetres = 5

Column Bed Length in centimetres = 35

Now,  $\pi = 3.14$   
Radius(R) =  $(5/2) = 2.5$   
Bed Length (L) = 35

$$\text{i.e., CV} = 3.14 * (2.5)^2 * 35 \\ = 686.875\text{ml}$$

### Calculations for C18

**Q). How to calculate packing material weight for 50mm column ID, with 350mm packing bed length?**

**A). Packing Material Weight (PMW) = Column Volume \* Packing Material Particle Size.**

**Note:** Particle size differs based on brands.

From the above example column volume = 686.875

Phenomenex c18 particle size is = 0.63

$$\begin{aligned} \text{i.e.,} \quad \text{PMW} &= 686.875 * 0.63 \\ &= 432.73 \text{ grams.} \end{aligned}$$

**Q). How to calculate solvent volume to make packing material slurry?**

**A). Solvent Volume for making slurry is nothing but, the column volume.**

**Example:** Solvent volume for 50mm ID column, with 350mm Packing bed length

$$\begin{aligned} \text{Now,} \quad \pi &= 3.14 \\ \text{Radius(R)} &= (5/2) = 2.5 \\ \text{Bed Length (L)} &= 35 \end{aligned}$$

$$\begin{aligned} \text{i.e., CV} &= 3.14 * (2.5)^2 * 35 \\ &= 686.875\text{ml.} \end{aligned}$$

**Q). What is the solvent to be used for slurry making?**

**A). For C18 packing material we have to choose IPA and Ethanol in 1:1 Ratio.**

**i.e., from the above example, 343.4375ml IPA and 343.4375ml Ethanol = 686.875ml.**

### Calculations for Silica:

**Q). How to calculate packing material weight for 50mm column ID, with 350mm packing bed length?**

**A). Packing Material Weight (PMW) = Column Volume \* Packing Material Particle Size.**

**Note:** Particle size differs based on brands.

From the above example column volume = 686.875

Phenomenex silica particle size is = 0.58

$$\begin{aligned} \text{i.e.,} \quad \text{PMW} &= 686.875 * 0.58 \\ &= 398.3875 \text{grams.} \end{aligned}$$

**Q). How to calculate solvent volume to make packing material slurry?**

**A). Solvent Volume for making slurry is nothing but, the column volume.**

**Example:** Solvent volume for 50mm ID column, with 350mm Packing bed length

$$\begin{aligned} \text{Now,} \quad \Pi &= 3.14 \\ \text{Radius(R)} &= (5/2) = 2.5 \\ \text{Bed Length (L)} &= 35 \end{aligned}$$

$$\begin{aligned} \text{i.e., CV} &= 3.14 * (2.5)^2 * 35 \\ &= 686.875 \text{ml.} \end{aligned}$$

**Q). what is the solvent to be used for silica slurry making?**

**A). For silica packing material we have to choose IPA and ACN in 1:1 Ratio.**

i.e., from the above example, **343.4375ml IPA and 343.4375ml ACN = 686.875ml.**

**Note:** It is must, that, water must not enter in to the silica packing material.