Application Note F2.0
Quick Overview of Column Chromatography

SiliaFlash®

1-METHODOLOGY

Before performing a separation on a chromatographic column, it is highly recommended to make different tests on thin layer chromatography (TLC) to find the best conditions for the separation. It is then important to use the same solvent system and adsorbent afterwards, on flash chromatography.

2-THEORY OF SEPARATION

For technique such as thin layer chromatography, it is not reliable to measure the retention of a compound as a function of the time but rather as a function of the distance. For this matter, the "retention factor", R_f, is defined by the ratio of the distance travelled by the analyte over the distance travelled by the solvent front. The difference between two R_f, namely ΔR_f, is a measure of the distance between analytes.

$$R_f = \frac{\text{Distance travelled by the analyte}}{\text{Distance travelled by the solvent front}}$$

$$\Delta R_f = R_{f1} - R_{f2}$$

Typically, the analyte of interest must have an R_f of 0.15-0.35 and a ΔR_f of at least 0.15 with the nearest compound, to obtain the best separation.

In flash chromatography separation is govern by column volumes (CV). This corresponds to the volume of solvent necessary to fill all the void volume in a packed column, i.e., sorbent pores and interstitial spaces between sorbent particles. The number of CV for a particular compound is the number of column volumes necessary to elute this compound. The correlation between the Rf and the CV is defined by:

$$CV = 1/R_f$$

The separation between two peaks, ΔCV is defined by:

$$\Delta CV = 1/R_{f1} - 1/R_{f2}$$

Ideally, the analyte of interest should have a CV between 3-6 and a ΔCV greater than 1 with the nearest compound.

3-CHOICE OF SOLVENT

Choice of the solvent, the mobile phase, is of crucial importance. The choice is guided by the polarity of the solvent depending of whether normal or reversed phase is used (low or high polarity respectively). Here is a list of different solvents and their relative strength:

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Solvent	Solvent strength
Methanol	0.95
Ethanol	0.88
2-propanol	0.82
acetonitrile	0.65
ethyl acetate	0.58
Tetrahydrofuran	0.57
Acetone	0.56
Dichloromethane	0.42
Chloroform	0.40
Diethyl ether	0.38
Toluene	0.29
Hexane	0.01

Combination of different solvents may be necessary to obtain a ΔR_f greater than 0.2. Also, mixtures of solvent of equivalent overall strength do not necessarily have the same selectivity. Acetonitrile does not have the same selectivity as methanol or ethanol. A mixture of 1:1 hexane/Ethyl acetate and 1:2 Hexane/dichloromethane may provide similar strength but may demonstrate different selectivity. Hence the trick is to try different combinations of solvents and solvent ratios.

4-LOADING THE COLUMN

The best method to fill a column with a sorbent is by the slurry method. In this technique, a container is filled with the least eluting solvent that is going to be used during the separation, non-polar or polar for normal or reversed phase respectively. The sorbent is then added to the solvent (and not the reverse) in order to make a slurry fluid enough so that it can be poured easily. The container is swirled to obtain an homogeneous solution and is rapidly poured into the column through a funnel. Quantities of sorbent added in this fashion should not exceed a layer of about 2 cm at the time. It is also important to tap gently the side of the column to improve packing. Drain excess solvent and make sure that the column never runs dry.

5-SOLVENT EQUILIBRATION

Before adding the sample, it is important to condition the column with the starting elution mixture, in order to obtain optimum and reproducible results. It can be done by washing the column with a volume of solvent 2 times that of the column volume. See the following table for details.

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Column Size (g)	Approximate Column Volume (mL)	Typical Solvent Volume (mL)
2	2.5	5
5	6.5	13
10	12.5	25
20	25	50
25	31.3	62.5
50	62.5	125
70	88	176
100	125	250
150	188	376

6-LOADING OF THE SAMPLE

Two different ways may be adopted: wet loading and dry loading.

Wet loading: This technique requires dissolving the sample to be purified in a minimum amount of solvent that has the least affinity with the sorbent. This means that non-polar solvent, such as hexane, should be used to load the sample in normal phase chromatography and that polar solvent, such as methanol, should be used to load the sample in reversed phase chromatography. The level of solvent in the column is brought down just to that of the sorbent. The dissolved sample is then added directly to the top of the column, dispensed evenly on the surface. Let the sample enter completely the column by lowering the level of liquid to the line of the sorbent. Rinse the side of the column with the same solvent and lower the level again to make sure that the entire sample is in contact with the sorbent.

Dry loading: This technique is recommended when the sample is not soluble enough in the prescribed solvent. Small quantities of a better solvent can be added to ensure complete dissolution. The sample is pre-adsorbed on a small quantity of sorbent, varying from a ratio sample/sorbent of 1:1 to 1:3 by volume. Solvent can be evaporated off on a rotary evaporator and the sample added above the top frit of the column. Finally, another frit may be placed atop. Press down this new surface to secure tight packing and prevent movement of the bed.

7-SAMPLE CAPACITY

Typically, 5 to 10% of mass sample relative to the column bed mass may be used for purification. Different factors such as analytes, concentration of reaction products, elution solvent used and sample matrix may affect the capacity of the column.

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