APPLICATION NOTE
#SF001-0

SiliCycle

SiliaFlash®

Quick Overview of Column Chromatography

Flash chromatography is a widely established chemical separation technique based on the polarity differences of compounds. To perform a successful purification, a method development is required to ensure the most efficient separation. This Application Note presents rules of thumb that can be followed in order to accomplish this goal.

LEARN MORE about Silia*Flash* in our brochure “Solutions For Purification & Chromatography.”

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Quick Overview of Column Chromatography

METHODOLOGY

Prior to performing a separation with a chromatographic column, it is highly recommended to perform screening tests using thin layer chromatography (*TLC*) to determine the best conditions for the separation. From the results obtained with the TLC plates, one can then use the same conditions (*solvent system and adsorbent*) when moving to flash chromatography.

THEORY OF SEPARATION

When using thin layer chromatography, retention of a compound is best measured as a function of distance rather than time. The "retention factor", R_f, is defined as 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.

$$\Delta R_f = R_{f2} - R_{f1} \qquad \text{Where } R_f = \frac{\text{Distance travelled by the analyte}}{\text{Distance travelled by the solvent front}}$$

Typically, the analyte of interest must have an R_f of 0.1 - 0.4 to obtain the best separation.

In flash chromatography, separation is governed by column volumes (*CV*), which corresponds to the volume of solvent necessary to fill all the void volume in a packed column. The void volume includes sorbent pores and interstitial spaces between sorbent particles. The number of column volumes necessary to elute a compound is that compound's CV number. The correlation between R_f and CV is defined as the following:

$$CV = \frac{1}{R_f}$$

The separation between two peaks, ΔCV, can be then defined as:

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

Ideally, the analyte of interest should have a CV between 2.5 - 10 and a ΔCV greater than 1 with the nearest compound.

CHOICE OF SOLVENT

The solvent (*aka the mobile phase*) is of crucial importance. Choosing the right solvent is dependent on its polarity and whether a normal phase (*low polarity*) or reversed-phase (*high polarity*) will be used. The table below lists possible solvents that can be used and their corresponding relative strength.

Table 1: Relative strength of solvents used in chromatography

Relative strength of solvents used in chromatography			
Solvent	**Solvent Strength**	**Solvent**	**Solvent Strength**
Methanol	0.95	Acetone	0.56
Ethanol	0.88	Dichloromethane	0.42
2-Propanol	0.82	Chloroform	0.40
Acetonitrile	0.65	Diethyl Ether	0.38
Ethyl Acetate	0.58	Toluene	0.29
Tetrahydrofuran	0.57	Hexane	0.01

A single solvent, or even a combination of solvents, can be used to obtain a ΔCV greater than 1. It is important to note that if using a combination of solvents with similar strengths, the solvent mix may have in fact a different resulting selectivity. Acetonitrile does not have the same selectivity as methanol or ethanol. For example, a mixture of 1:1 hexane : ethyl acetate or 1:2 hexane : dichloromethane may provide a similar strength but may demonstrate different selectivities. Hence the trick is to try different combinations of solvents and solvent ratios.

CHOICE OF STATIONARY PHASE

SiliCycle offers one of the most reliable portfolios for flash and gravity grades for low to medium-high pressure:

Table 2: Available formats: from 1 kg to 25 kg, even up to multi-ton scale!

Silia*Flash* Irregular Silica Gels Portfolio			
Product Number	Partical Size		Pore Diameter (Å)
	µm	Mesh	
R10137L	75-150	100-200	30
R10130A	40-63	230-400	40
R10150A	60-120	325-625	
R10140A	60-200	70-230	
R10160A	120-200	70-125	
R10170A	200-500	35-70	
R10180A	500-1000	18-35	
R10117B	15-40	*	60
R10023B	20-45	*	
R10030B(*F60*)	40-63	230-400	
R12030B(*P60*)			
R10530B(*Acid-Washed*)			
R10150B	60-120	325-625	
R10040B(*G60*)	60-200	70-230	
R10140B(*GE60*)			
R10137B	75-150	100-200	
R10157B	105-175	86-140	
R10160B	120-200	70-125	
R10165B	150-250	60-100	
R10170B	200-500	35-70	
R10180B	500-1000	18-35	

Silia*Flash* Irregular Silica Gels Portfolio			
Product Number	Partical Size		Pore Diameter (Å)
	µm	Mesh	
R10157D	105-175	86-140	90
R10170D	200-500	35-70	
R10180D	500-1000	18-35	
R10181D	800-1200	16-22	
R10130H	40-63	230-400	150
R10150H	60-120	325-625	
R10140H	75-250	70-200	
R10157H	105-175	86-140	
R10160H	120-200	70-125	
R10170H	200-500	35-70	
R10072H	250-500	35-60	
R10180H	500-1000	18-35	
R10181H	800-1200	16-22	
R10130M	40-63	230-400	300
R10140M	60-200	70-230	
R10170M	200-500	35-70	

* Mesh equivalent too small to exist as real screen size

PACKING THE COLUMN

The best method to fill a column with a sorbent is by what is called the slurry method. In this method, a beaker is first filled with the least eluting solvent planned for the separation, either a non-polar one for normal phase or a polar solvent for reversed-phase. The sorbent is then added to the solvent, and not other way around, in order to make a slurry fluid enough to be poured easily. Once a homogenous mixture is obtained, it can be rapidly poured into the column with the help of a funnel. Quantities of sorbent added in this fashion should not exceed a layer of about 2 cm at a time. It is also important to tap gently the side of the column to improve packing. The excess solvent should be drained until it arrives to the sorbent level. Care must be given to not dry out the column.

SOLVENT EQUILIBRATION

Conditioning the column with the starting elution mixture should be done before adding the sample, for optimum and more reproducible results. This is done by washing the sorbent with 2 column volumes (*2 CV*).

Table 3: Column and solvents volumes according to column size

Column and Solvents Volumes According to Column Size		
Column Size (*g*)	Approximate Column Volume (*mL*)	Typical Solvent Volume (*mL*)
2	3	6
5	7	14
10	13	26
20	25	50
25	32	64
50	63	126
70	88	176
100	125	250
150	188	376

LOADING THE SAMPLE

Wet Loading

Wet loading refers to the technique of dissolving the sample to be purified in a minimum amount of solvent; the weakest solvent possible. Meaning that a non-polar solvent should be used to load the sample in normal phase chromatography, and that a polar solvent 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 subsequently added directly to the top of the column, dispensed evenly on the surface. Next, 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

Dry loading is recommended when the sample is not soluble enough in a weak solvent. Small quantities of a stronger solvent can be added to ensure complete dissolution. The sample is pre-adsorbed on a small quantity of sorbent. Varying ratios of sample : sorbent can be used, from 1:1 to 1:3 by volume. Solvent can be evaporated off on a rotary evaporator and the sample-sorbent mix is then added on top of the column. Press down to ensure tight packing and prevent movement of the bed.

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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