Full of Theories and Techniques

of Preparative Chromatography

You Need to Know

Biotage

www.biotage.co.jp

Biotage SNAP Column

Dragon Volume

Long-awaited second edition

Principle and application of

chromatography

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1. Theory You Need to Know

Five-Minute Principle of Flash Chromatography

1.1. Adsorption on Silica Gel

Silica gel is extensively used for purification by flash chromatography. Although it is well known that compounds adsorb on silica gel, much attention is not paid to the principle. Unlike the adsorption of compounds onto pores of activated carbon, adsorption is based on the interaction among the surface of silica gel (hydroxyl group [-OH] for normal-phase, octadecyl group [C18] for reverse-phase), compounds, and the mobile phase.

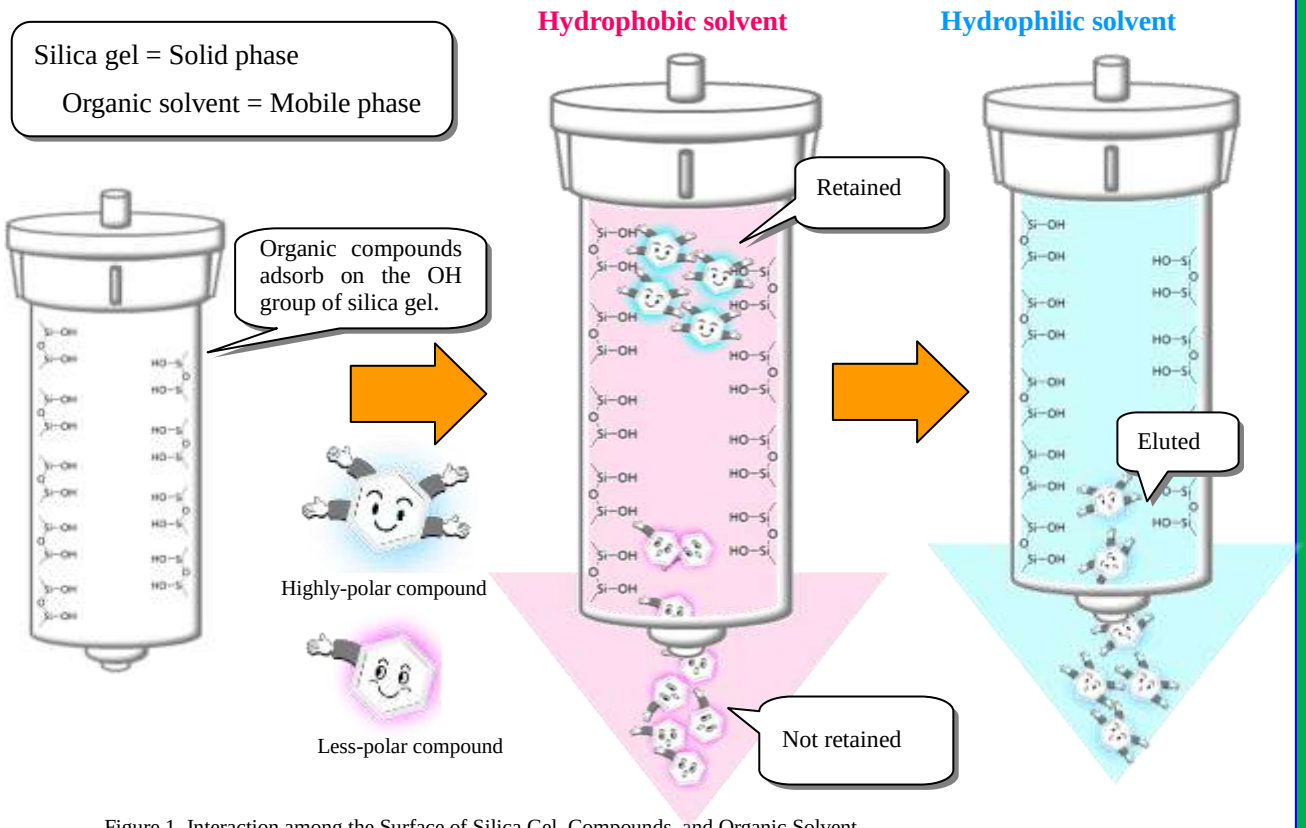


Figure 1. Interaction among the Surface of Silica Gel, Compounds, and Organic Solvent.

1.2. Mobile Phase

Silica gel is called the solid phase, whereas liquid flowing through the column is called the mobile phase. For chromatography, the mobile phase consisting of hydrophobic solvent and hydrophilic solvent is generally used.

For example, when a combination of ethyl acetate and hexane is used as the mobile phase in normal-phase chromatography, hydrophobic compounds are eluted from a column with a low concentration of ethyl acetate (hydrophilicity), and hydrophilic compounds are detached from the silica gel with an increasing concentration of ethyl acetate.

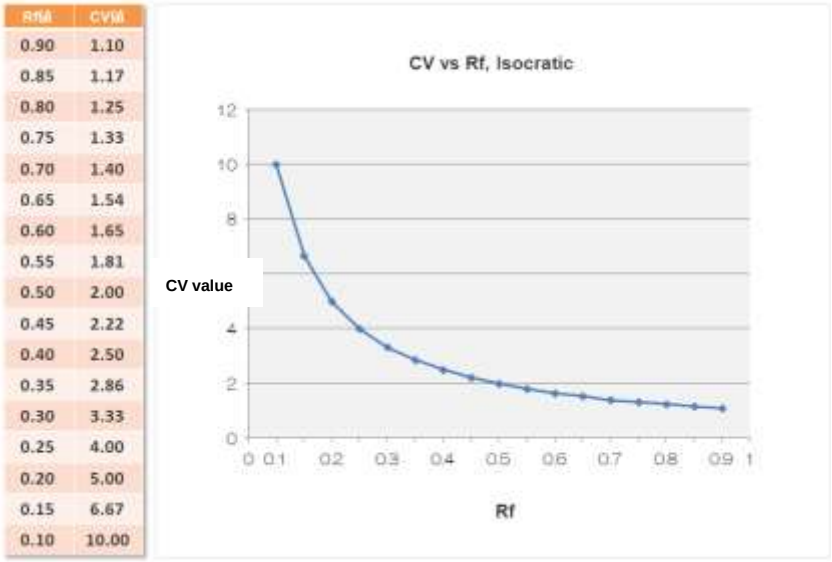
However, attention should be paid to hydrophobic and hydrophilic interactions as well as the influence of solvent properties on elution. For example, the use of dichloromethane (DCM) may produce completely different chromatograms.

1.3. Column Volume

The column volume indicates a void space in a silica gel-packed column. The volume of the mobile phase required to fill the void space is called one column volume. Chromatography may be theoretically described on the basis of the column volume.

1.4. Relationship between the Rf Value and the Column Volume (CV) Value

The column volume is an absolute value that depends on column size, and the CV value is a relative value that is defined as the reciprocal of an Rf value on TLC (CV value = 1 ÷ Rf value). The use of the CV value, a relative value, helps you understand chromatography. This section details the CV value.



Rf	CV
0.90	1.10
0.85	1.17
0.80	1.25
0.75	1.33
0.70	1.40
0.65	1.54
0.60	1.65
0.55	1.81
0.50	2.00
0.45	2.22
0.40	2.50
0.35	2.86
0.30	3.33
0.25	4.00
0.20	5.00
0.15	6.67
0.10	10.00

Figure 2. Correlation between the Rf Value and the CV Value.

Figure 2 shows the correlation between the Rf value and the CV value. For example, a compound with an Rf value of 0.5 has a CV value of 2 and is eluted with two column volumes. Elution of a compound with an Rf value of 0.2 requires 5 CV. Because CV values represent the behaviors of compounds in a flash column, identification of the CV value of a compound of interest is very useful for column pretreatment and fractionation.

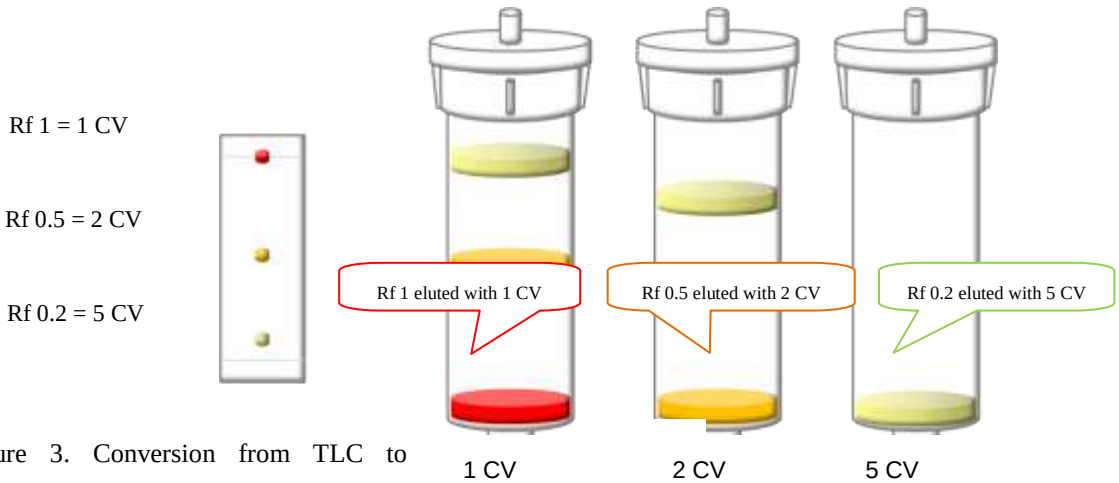


Figure 3. Conversion from TLC to Column Chromatography.

1.5. Prediction of Chromatograms from Rf Values

When predicting chromatograms from Rf values, people tend to attach importance to the distance between spots. In TLC, the solvent ratio that maximizes the distance provides the best condition for separation. However, the condition cannot be used for flash chromatography. When TLC spots are located close to the original spot, the distance between peaks is large in flash chromatography.

Samples:

1-Nitronaphthalene	0.2 mg
o-Nitroaniline	0.2 mg
m-Nitroaniline	0.2 mg
p-Nitroaniline	0.2 mg

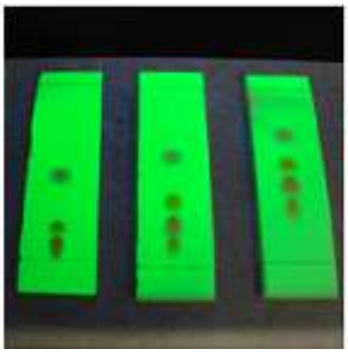
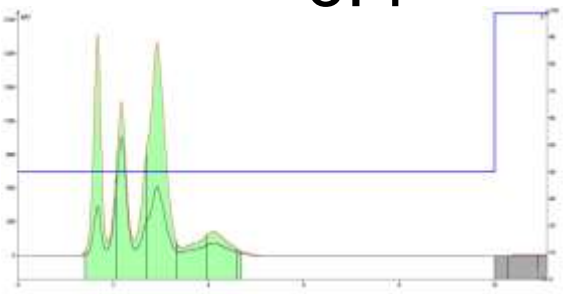
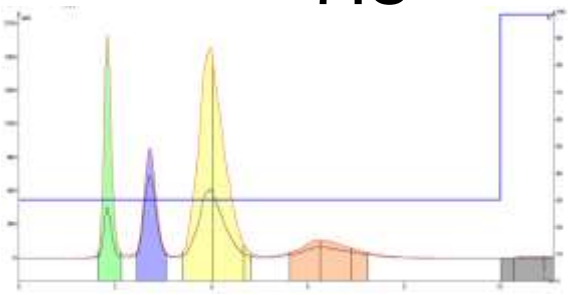


Figure 4. Relationship between Rf and Chromatography.

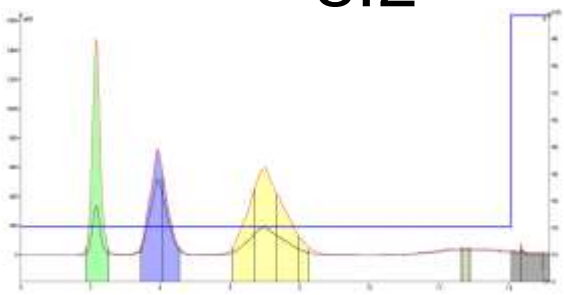
Chromatogram 1.
Hexane/Ethyl Acetate 6:4



Chromatogram 2.
Hexane/Ethyl Acetate 7:3



Chromatogram 3.
Hexane/Ethyl Acetate 8:2



You may think the mixture ratio of 6:4 appropriate because spots are well distributed on the TLC plate in Figure 3. As shown in Chromatogram 1, however, satisfactory separation is not achieved. When spots are too close to the origin, samples are diffusely separated from each other, and the fourth peak is not visible as shown in Chromatogram 3. Thus, it is difficult to predict chromatograms from Rf values alone.

For satisfactory separation, it is important to modify the composition of mobile phase and adjust the Rf value of analytes in the range of 0.15 to 0.35.

1.6. Prediction of Chromatograms from CV Values

Chromatograms are reasonably predicted from CV values. The CV value calculated from TLC results may be used to calculate the elution time of compounds and the volume of solvent required for purification. An example of the use of an SNAP 25-g Cartridge is provided below.

SNAP 25 g: 1 CV = 33 ml

Compound A: Rf 0.55 = CV value 1.74 = Peak top is observed after 57.4 ml.

Compound B: Rf 0.35 = CV value 2.86 = Peak top is observed after 94.38 ml.

Compound C: Rf 0.20 = CV value 5.00 = Peak top is observed after 165 ml.

Compound D: Rf 0.13 = CV value 8.00 = Peak top is observed after 264 ml.

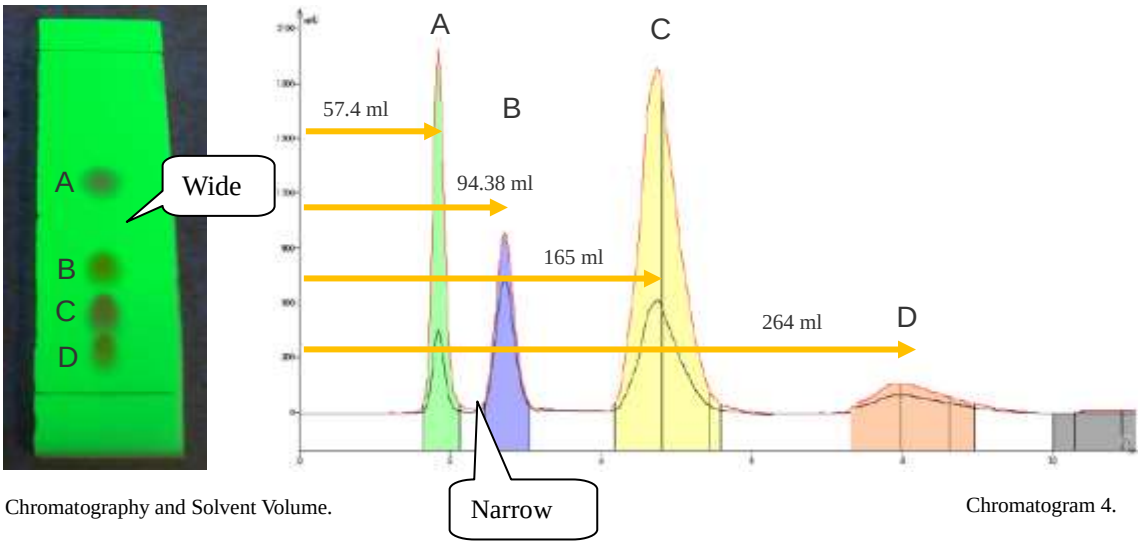


Figure 5. Chromatography and Solvent Volume.

Chromatogram 4.

1.7. Gradient Formation

Anyone can readily form a gradient by following a certain rule.

[First step toward gradient formation]

1) 1 CV at the starting concentration that is one fourth the TLC solvent ratio
2) 10 CV from the starting concentration to the final concentration that is twice the TLC solvent ratio
3) 2 CV at the final concentration

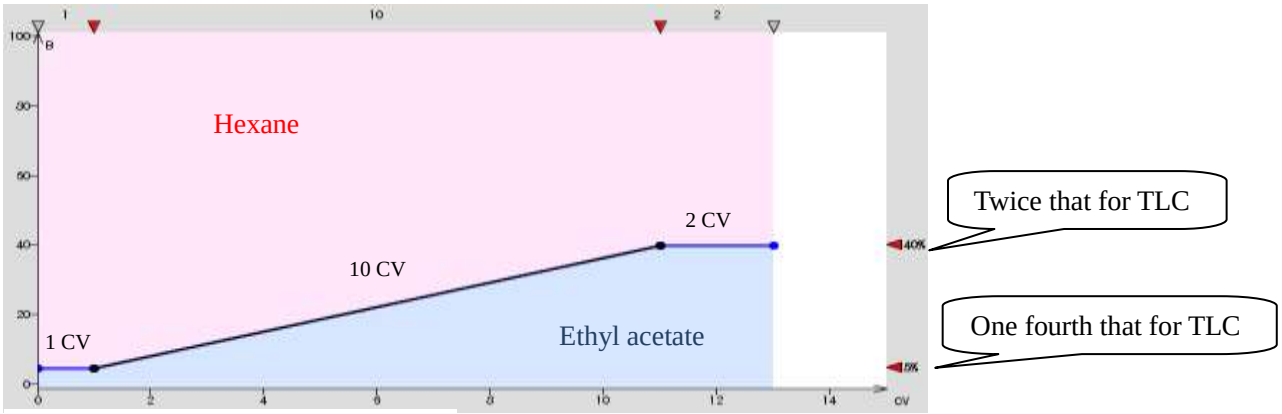


Figure 6. Example of Gradient Formation.

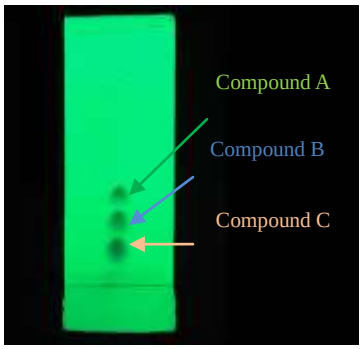


Figure 7. TLC with a Solvent Ratio of 15:1.

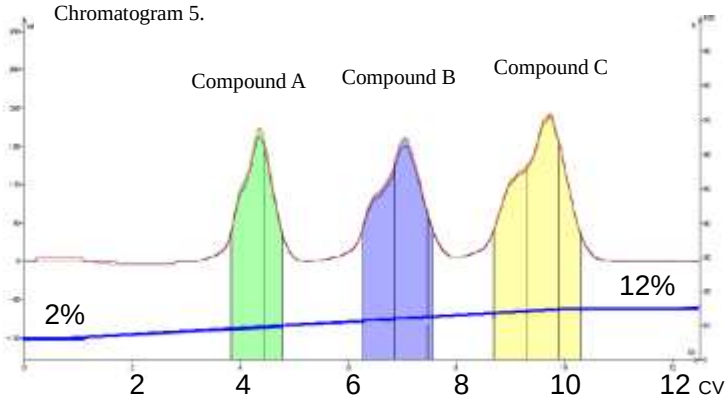


Figure 7 shows a TLC developed with hexane/ethyl acetate ratio of 15:1. In Chromatogram 5, the starting concentration of gradient was 2% (one fourth the TLC solvent ratio [ethyl acetate concentration of 6.25%]), and the final concentration was 12% (twice the TLC solvent ratio).

ADVANCED TIP

The setting of step gradient with highly polar solvent as described below reduces the purification time and the volume of solvent used. The same samples used in Figure 7 were separated by step gradient.

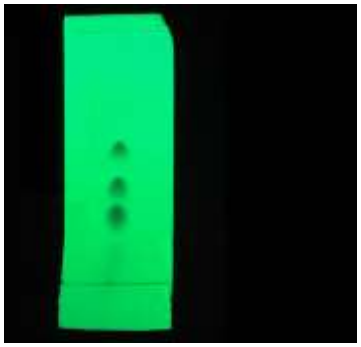
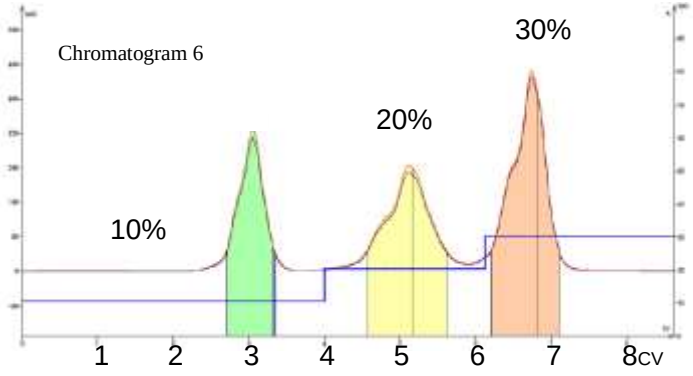


Figure 8. TLC with a Solvent Ratio of 9:1.



In Figure 8, TLC was developed with a solvent ratio (hexane: ethyl acetate) of 9:1 for accelerated elution. Compound A with an Rf value of 0.45 should be eluted with 2.2 CV when the concentration of ethyl acetate is 10%. Compound B with an Rf value of 0.25 is expected to be eluted with 4 CV or more. Thus, if the concentration is maintained at 10% for the first 4 CV, compound A can be clearly separated from compounds B and C. A stepwise increase in the concentration to 20% allows elution of compound B, and then an increase in the ethyl acetate concentration to 30% allows quick elution of the final compound. This approach resulted in a 30% reduction in solvent volume and separation time (see Chromatogram 6).

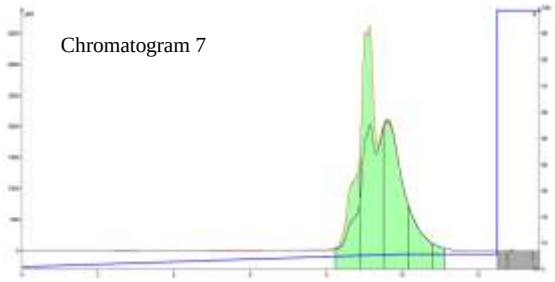
2. Practice You Need to Know Quick, Useful Techniques

2.1 Pretreatment for Halogen/Methanol Separation "What is an Interfering Material?"

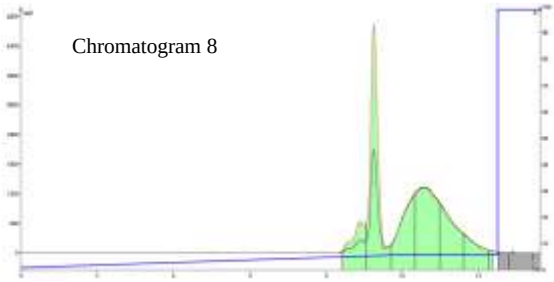
Water often interferes with chromatographic separation, which is not limited to the use of halogenated solvent and methanol. In particular, poor separation with the use of halogenated solvent and methanol may be overcome by modification of pretreatment procedures. This phenomenon results from inappropriate adjustment of the pH on the surface of silica gel or water adhering to the surface of silica gel. Passage of the hydrophilic solvent-containing mobile phase through the column as a pretreatment may help adjust the pH and remove water.

POINT:

Water adhering to silica gel cannot be removed by pretreatment with hydrophobic solvent alone.
Water is successfully removed by passage of at least 3 CV of solvent containing hydrophilic solvent (generally at least 3% or the starting concentration of gradient).



Chromatogram after pretreatment with hydrophobic solvent alone



Chromatogram after pretreatment with hydrophobic and hydrophilic solvents

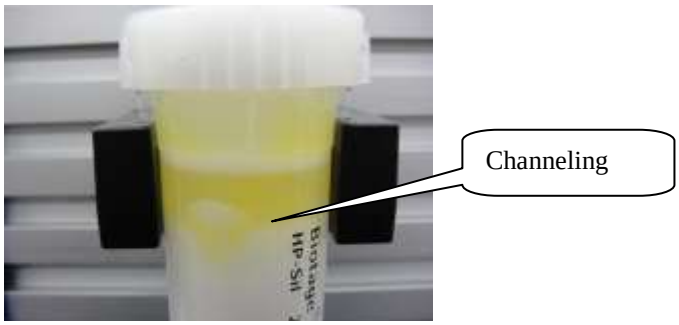
Samples: 0.2 mg each of promethazine chloride and 4-(dimethylamino) antipyrine

2.2 Considerations in Halogen/Methanol Separation

In halogen/methanol separation, attention should be paid to the time of exposure of the column to solvent. Polypropylene, a component of the cartridge, absorbs halogenated solvents, such as dichloromethane (DCM) and swell. Longer exposure may cause problems with silica gel filling and sample channeling.* When halogenated solvent is used, pretreatment should be followed immediately by sample charge and chromatographic separation.

*An empty space between silica and housing results in poor band shape.

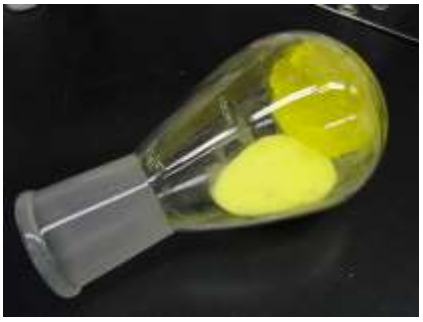
Band formed when chromatography was run immediately after pretreatment.



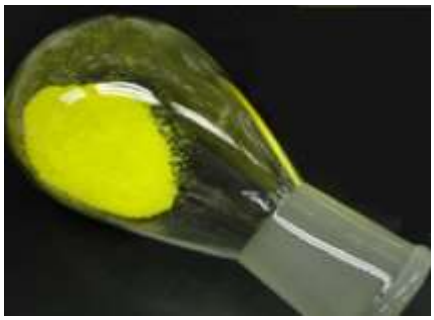
Band formed when chromatography was run 1 hour after pretreatment.

2.3 Preadsorption Technique (poorly soluble samples are adsorbed before loading)

A technique is available in which poorly soluble samples are adsorbed on silica gel and then applied onto a column. When the amount of silica gel is small, samples are not uniformly adsorbed but agglutinated. The use of a large amount of silica gel for prevention of agglutination may result in diffuse bands and poor separation. In this case, the use of diatomaceous earth is recommended instead of silica gel.

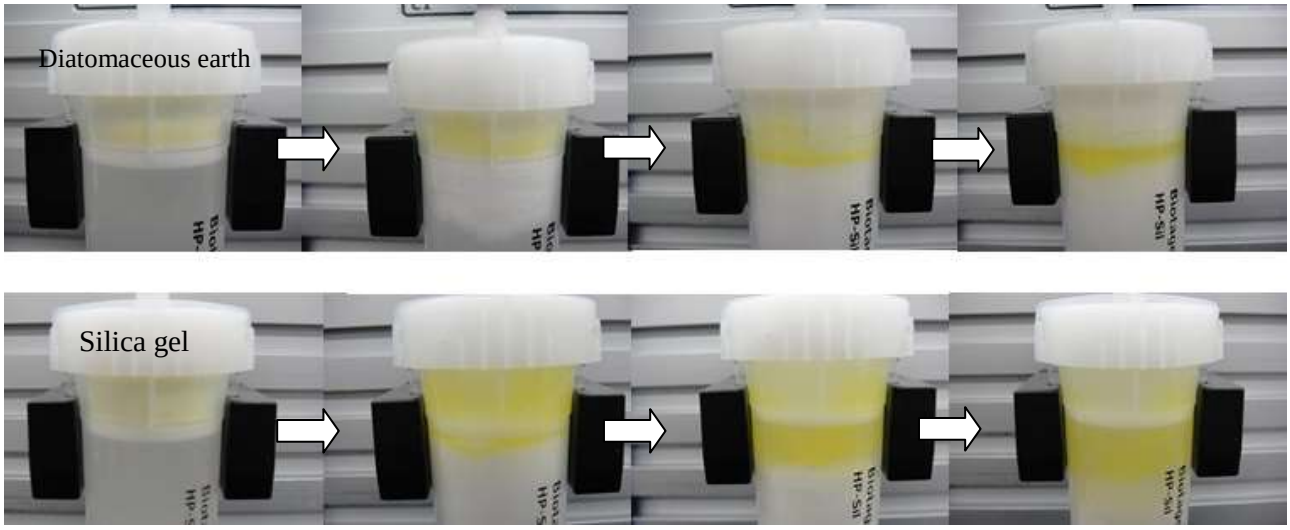


Preadsorption on silica gel (3 g)



Preadsorption on diatomaceous earth (3 g)

Unlike silica gel, diatomaceous earth has no interaction with samples and prevents band diffusion. Because diatomaceous earth is hygroscopic, a small amount is enough for sample preadsorption.

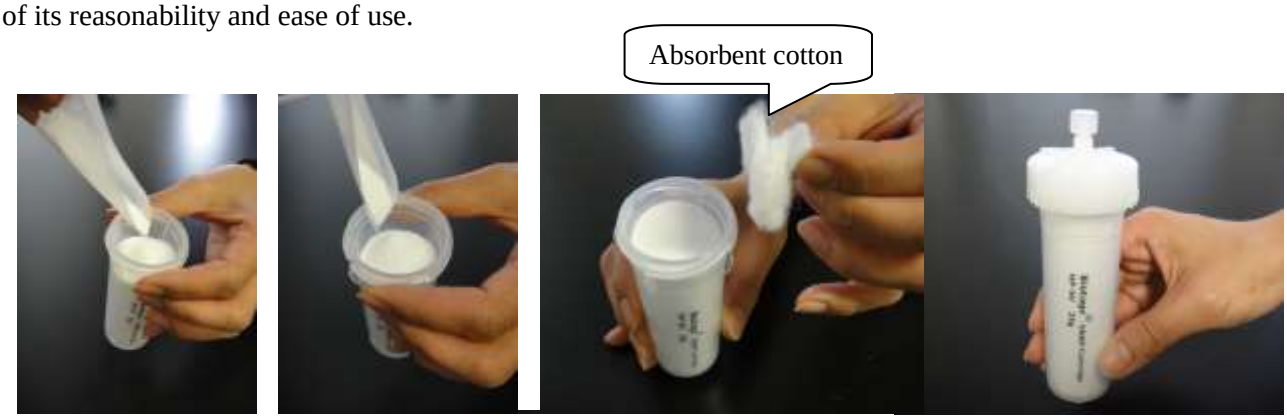


Tip:

Parts Number	Description	Qty/cs	Japan List
Diatomaceous earth bulk			
9800-5000	ISOLUTE HM-N BULK 5KG	1	54,500
9800-1000	ISOLUTE HM-N BULK 1KG	1	12,600
9800-0500	ISOLUTE HM-N BULK 500g	1	8,200

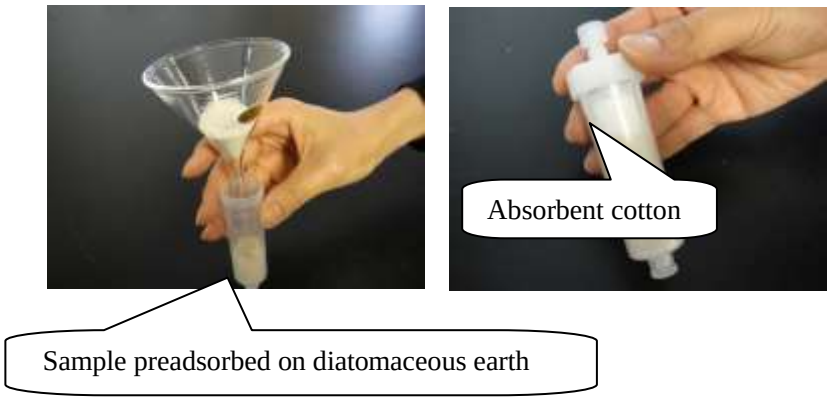
2.4 Backflow Prevention with Absorbent Cotton

A preadsorbed sample may be packed on the upper part of a SNAP Cartridge. In this case, absorbent cotton is useful in preventing the backflow of silica gel. Absorbent cotton is a recommended item because of its reasonability and ease of use.



2.5. Sample Charge with an Empty Column

Biotage offers SNAP Empty Cartridges. The use of an empty column facilitates packing of a preadsorbed sample. The Luer-lock joint allows direct connection to a main cartridge.



3. Ordering Information

Part Number	Description	Qty/cs	Japan List
High-Performance Silica Gel Cartridge (20 µm)			
FSHP-1207-0010	SNAP 10g High-Performance Silica	20	21,700
FSHP-1207-0025	SNAP 25g High-Performance Silica	20	32,000
FSHP-1207-0050	SNAP 50g High-Performance Silica	20	54,000
FSHP-1207-0100	SNAP 100g High-Performance Silica	20	95,000
FSHP-1207-0340	SNAP 340g High-Performance Silica	6	95,000
Normal Silica Gel Cartridge (50 µm)			
FSK0-1107-0010	SNAP KP-Sil Cartridge, 10 g	20	18,600
FSK0-1107-0025	SNAP KP-Sil cartridge, 25 g	20	26,000
FSK0-1107-0050	SNAP KP-Sil Cartridge, 50 g	20	44,100
FSK0-1107-0100	SNAP KP-Sil Cartridge, 100 g	20	62,000
FSK0-1107-0340	SNAP KP-Sil cartidge, 340g	6	67,800
Amino-Modified NH-Silica Gel Cartridge			
TLC-KPNH-2510-FI	KP-NH TLC plates, 10x10cm snap apart	25	48,400
FSN0-0909-0011	SNAP KP-NH cartridge, 11g	10	25,000
FSN0-0909-0028	SNAP KP-NH cartridge, 28g	10	40,000
FSN0-0909-0055	SNAP KP-NH cartridge, 55g	10	59,000
FSN0-0909-0110	SNAP KP-NH cartridge, 110g	10	75,000
FSN0-0909-0375	SNAP KP-NH cartridge, 375g	1	29,500
Reverse-Phase C18 Cartridge			
FSL0-1118-0012	SNAP KP-C18-HS cartridge, 12g	2	8,000
FSL0-1118-0030	SNAP KP-C18-HS cartridge, 30g	2	18,000
FSL0-1118-0060	SNAP KP-C18-HS cartridge, 60g	2	30,000
FSL0-1118-0120	SNAP KP-C18-HS cartridge, 120g	2	50,000
FSL0-1118-0400	SNAP KP-C18-HS cartridge, 400g	1	80,000
Samplet High-Performance Silica Gel			
SAS-1207-0010	SNAP Samplet 1g High-Performance Silica	20	15,000
SAS-1207-0025	SNAP Samplet 3g High-Performance Silica	20	16,700
SAS-1207-0100	SNAP Samplet 10g High-Performance Silica	20	33,000
SAS-1207-0340	SNAP Samplet 34g High-Performance Silica	6	33,000
Samplet Normal Silica Gel			
SAS-1107-0010	SNAP KP-Sil Samplet, 1 g	20	10,300
SAS-1107-0025	SNAP KP-Sil Samplet, 3g	20	14,000
SAS-1107-0100	SNAP KP-Sil Samplet, 10 g	20	22,100
SAS-1107-0340	SNAP KP-Sil Samplet, 34g	6	24,200
Samplet Amino-Modified Silica Gel			
SAS-0909-0011	SNAP KP-NH Samplet, 1g	20	9,200
SAS-0909-0028	SNAP KP-NH Samplet, 3g	20	15,000
SAS-0909-0110	SNAP KP-NH Samplet, 11g	20	38,000
SAS-0909-0375	SNAP KP-NH Samplet, 37g	6	45,000
Samplet C18			
SAS-1118-0012	SNAP KP-C18-HS Samplet, 1g	20	11,000
SAS-1118-0030	SNAP KP-C18-HS Samplet, 3g	20	28,000
SAS-1118-0120	SNAP KP-C18-HS Samplet, 12g	20	66,000
SAS-1118-0400	SNAP KP-C18-HS Samplet, 40g	6	65,000
Empty Cartridge			
SEC-0010	SNAP Empty cartridge kit for Injection, 10g, 20/cs	20	9,000
SEC-0025	SNAP Empty cartridge kit for Injection, 25g, 20/cs	20	12,500
SEC-0050	SNAP Empty cartridge kit for Injection, 50g, 20/cs	20	24,000
SEC-0100	SNAP Empty cartridge kit for Injection, 100g, 20/cs	20	36,000
Empty Samplet			
SES-0010	SNAP empty Samplet kit, 1g	20	7,600
SES-0025	SNAP empty Samplet kit, 3g	20	8,300
SES-0100	SNAP empty Samplet kit, 10g	20	9,000
SES-0340	SNAP 340g empty Samplet kit, contains emptly Samplet w/ bottom frits (6), top frits (6), and a frit insertion tool (1)	6	10,400

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