



Technical Tips

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Solvent miscibility table & cut-off

UV (nm) Cutoff	Viscosity (CPoise) 20°C	Solvent	Acetone	Acetonitrile	Dimethylformamide	Dimethylsulfoxyde	1,4-Dioxane	Ethanol	Isopropanol	Methanol	Tetrahydrofurane	Water	Benzene	n-Butanol	Carbone Tetrachloride	Chloroform	Cyclohexane	1,2-Dichloroethane	Dichloromethane	Ethyl Acetate	Diethyl ether	Heptane	Hexane	Iso-octane	Methyl tert-butyl ether	Butanone	Pentane	Toluene	Xylene
330	0.36	Acetone	Green																										
190	0.38 (15°C)	Acetonitrile	Green	Green																									
268	0.92	Dimethylformamide	Green	Green	Green																								
268	2.24	Diméthylsulfoxyde	Green	Green	Green	Green																							
215	1.37	1,4-Dioxane	Green	Green	Green	Green	Green																						
210	1.20	Ethanol	Green	Green	Green	Green	Green	Green																					
120	2.30	Isopropanol	Green	Green	Green	Green	Green	Green	Green																				
205	0.59	Methanol	Green	Green	Green	Green	Green	Green	Green	Green																			
215	0.55	Tetrahydrofurane	Green	Green	Green	Green	Green	Green	Green	Green	Green																		
200	1.00	Water	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green																
280	0.65	Benzene											Dark Blue																
254	0.73	n-Butanol											Dark Blue																
263	0.97	Carbone Tetrachloride											Dark Blue																
245	0.57	Chloroform											Dark Blue																
200	1.00	Cyclohexane											Dark Blue																
225	0.79	1,2-Dichloroethane											Dark Blue																
235	0.44	Dichloromethane											Dark Blue																
260	0.45	Ethyl Acetate											Dark Blue																
220	0.32	Diethyl ether											Dark Blue																
200	0.42	Heptane											Dark Blue																
200	0.31	Hexane											Dark Blue																
215	0.50	Iso-octane											Dark Blue																
210	0.27	Methyl tert-butyl ether											Dark Blue																
329	0.45	Butanone											Dark Blue																
200	0.23	Pentane											Dark Blue																
285	0.59	Toluene											Dark Blue																
290	0.61	Xylene											Dark Blue																

Green Miscible with water

White Miscible

Dark Blue Immiscible

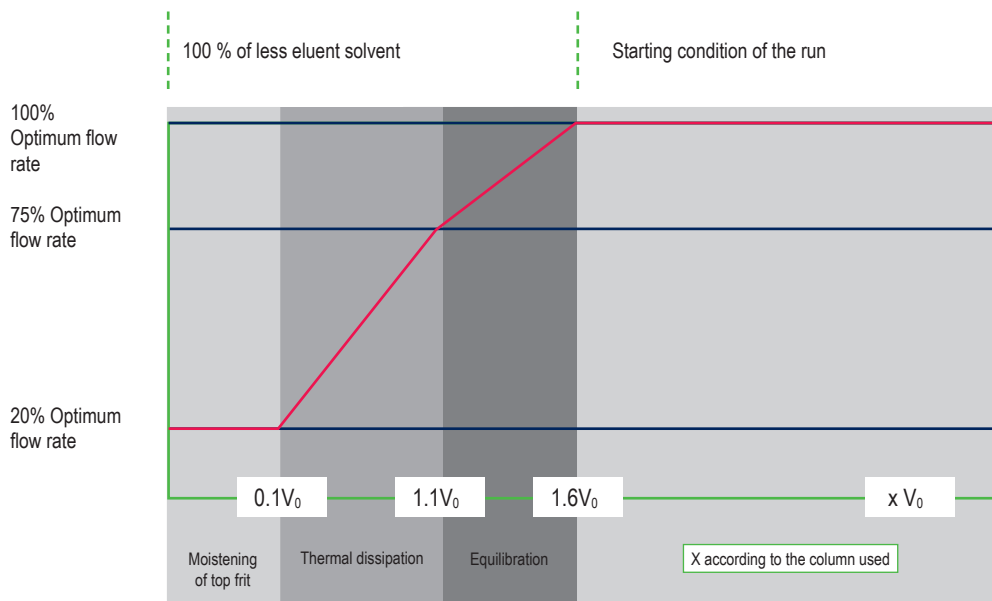


Solvent Strength

Solvents List	ξ_0 Silica Virgin	ξ_0 Alumina	ξ_0 Silica Diol	ξ_0 Silica CN	ξ_0 Silica NH2	ξ_0 Silica C18, C4, C8, PH, RPAQ	ξ_0 Magnesie	ξ_0 Florisil
Acetone	0.470	0.560	0.141	0.470	0.470		0.325	0.291
Acetonitrile	0.501	0.650	0.150	0.501	0.501	0.577	0.377	0.338
Benzene	0.246	0.319	0.074	0.246	0.246		0.185	0.166
Butanol	0.550	0.714	0.165	0.550	0.550		0.414	0.371
Carbon tetrachloride	0.139	0.180	0.042	0.139	0.139		0.104	0.094
Chloroform	0.260	0.400	0.078	0.260	0.260		0.232	0.208
Cyclohexane	0.030	0.040	0.000	0.000	0.000		0.023	0.021
Cyclopentane	0.000	0.050	0.000	0.000	0.000		0.029	0.026
1,2-Dichloroethane	0.339	0.490	0.102	0.339	0.339		0.284	0.255
Dichloromethane	0.323	0.420	0.097	0.323	0.323		0.244	0.218
Diethylamine	0.485	0.630	0.146	0.485	0.485		0.365	0.328
Diethyl ether	0.385	0.380	0.115	0.385	0.385		0.220	0.198
Diisopropyl ether	0.223	0.280	0.067	0.223	0.223		0.162	0.146
N,N-Dimethylformamide	0.640	0.831	0.192	0.640	0.640		0.482	0.432
Dimethyl sulfoxide	0.470	0.620	0.141	0.470	0.470		0.360	0.322
Dioxane	0.490	0.560	0.147	0.490	0.490		0.325	0.291
Ethanol	0.677	0.879	0.203	0.677	0.677		0.510	0.457
Ethyl acetate	0.380	0.580	0.114	0.380	0.380		0.336	0.302
Heptane	0.000	0.000	0.000	0.000	0.000		0.000	0.000
Hexane	0.000	0.010	0.000	0.000	0.000		0.006	0.005
Hexanol	0.385	0.500	0.115	0.385	0.385		0.290	0.260
Isooctane	0.000	0.010	0.000	0.000	0.000		0.006	0.005
Isopropanol	0.590	0.820	0.177	0.590	0.590		0.476	0.426
Isopropyl chloride	0.223	0.290	0.067	0.223	0.223		0.168	0.151
Methanol	0.732	0.950	0.219	0.732	0.732	0.450	0.551	0.494
Methyl acetate	0.393	0.510	0.118	0.393	0.393		0.296	0.265
Methyl ethyl ketone	0.393	0.510	0.118	0.393	0.393		0.296	0.265
Methyl tert-butyl ether	0.470	0.610	0.141	0.470	0.470		0.354	0.317
Pentane	0.000	0.000	0.000	0.000	0.000		0.000	0.000
Petroleum ether	0.000	0.010	0.000	0.000	0.000		0.006	0.005
Propanol	0.631	0.819	0.189	0.631	0.631		0.475	0.426
Pyridine	0.550	0.714	0.165	0.550	0.550		0.414	0.371
Tetrahydrofuran	0.346	0.449	0.104	0.346	0.346	0.726	0.261	0.234
Toluene	0.223	0.290	0.067	0.223	0.223		0.168	0.151
Water						0.000		



Flash columns conditioning (from F0001 up to F1600 format)



Equilibration volume per column

	Format	V ₀ (mL)	nb of V ₀
IR-50SI PF-50SIAG PF-ALN PF-ALB	F0001	2.2	5
	F0004	5.2	5
	F0012	21.3	4
	F0025	33.6	4
	F0040	54.0	4
	F0080	113.6	4
	F0120	165.9	3
	F0220	304.0	3
	F0330	456.7	3
	F0800	1174.6	2
F1600	2333.4	2	
IR-20SI PF-50SIHP	F0004	5.2	5
	F0012	21.1	4
	F0025	33.2	4
	F0040	53.3	4
	F0080	112.2	4
	F0120	163.9	3
	F0220	300.4	3
	F0330	451.0	3
	F0800	1159.6	2
	F1600	2303.4	2
PF-30SIHP	F0004	5.1	4
	F0012	20.9	3
	F0025	32.8	3
	F0040	52.7	3
	F0080	110.8	3
	F0120	161.8	2
	F0220	296.8	2
	F0330	445.4	2
	F0800	1144.7	2
	F1600	2273.4	2

	Format	V ₀ (mL)	nb of V ₀
PF-50SIHC	F0004	5.4	4
	F0012	22.0	3
	F0025	34.8	3
	F0040	55.8	3
	F0080	117.8	3
	F0120	172.1	2
	F0220	314.8	2
	F0330	473.6	2
	F0800	1219.6	2
	F1600	2423.3	2
PF-25SIHC	F0004	5.4	4
	F0012	21.8	3
	F0025	34.4	3
	F0040	55.2	3
	F0080	116.4	3
	F0120	170.0	2
	F0220	311.2	2
	F0330	468.0	2
	F0800	1204.6	2
	F1600	2393.3	2
PF-15SIHC	F0001	2.2	5
	F0004	5.3	4
	F0012	21.6	3
	F0025	34.0	3
	F0040	54.6	3
	F0080	115.0	3
	F0120	168.0	2
	F0220	307.6	2
	F0330	462.3	2



	Format	V0 (mL)	nb of V0
PF-15SIHP	F0001	2.1	5
	F0004	5.0	4
	F0012	20.7	3
	F0025	32.4	3
	F0040	52.1	3
	F0080	109.4	3
	F0120	159.8	2
	F0220	293.2	2
	F0330	439.7	2

IR-50C18			
PF-C18HQ			
PF-C18XS			
PF-C18HP			
PF-C18AQ			
PF-RPAQ			
PF-PHC4	F0001	2.2	5
PT-C18T	F0004	5.2	5
PT-C8	F0012	21.3	5
PT-C4	F0025	33.6	5
PP-C18	F0040	54.0	5
PP-C4	F0080	113.6	5
PT-C18XS	F0120	165.9	5
PT-C18N	F0220	304.0	5
PT-C8N	F0330	456.7	5
PT-C18AQ	F0800	1174.6	5
PP-C4AQ	F1600	2333.4	5
PFB-C18N			
PFB-C18T			
PFB-C18XS			
PT-RP			
PP-RPT			

	F0004	5.2	5
PF-DIOL	F0012	21.3	5
PF-MM1	F0025	33.6	5
PF-CN	F0040	54.0	5
PF-NH2HC	F0080	113.6	5
PF-NH2	F0120	165.9	5
PF-SAX	F0220	304.0	5
PF-SCX	F0330	456.7	5
	F0800	1174.6	5
	F1600	2333.4	5

	F0001	2.2	6
	F0004	5.2	6
	F0012	21.3	6
	F0025	33.6	6
PF-15HIA	F0040	54.0	6
	F0080	113.6	6
	F0120	165.9	6
	F0220	304.0	6
	F0330	456.7	6

	Format	V0 (mL)	nb of V0
PF-X PF-100P6	F0004	5.2	5
	F0012	21.3	5
	F0025	33.6	5
	F0040	54.0	5
	F0080	113.6	5
	F0120	165.9	5
	F0220	304.0	5
	F0330	456.7	5
	F0800	1174.6	5
	F1600	2333.4	5

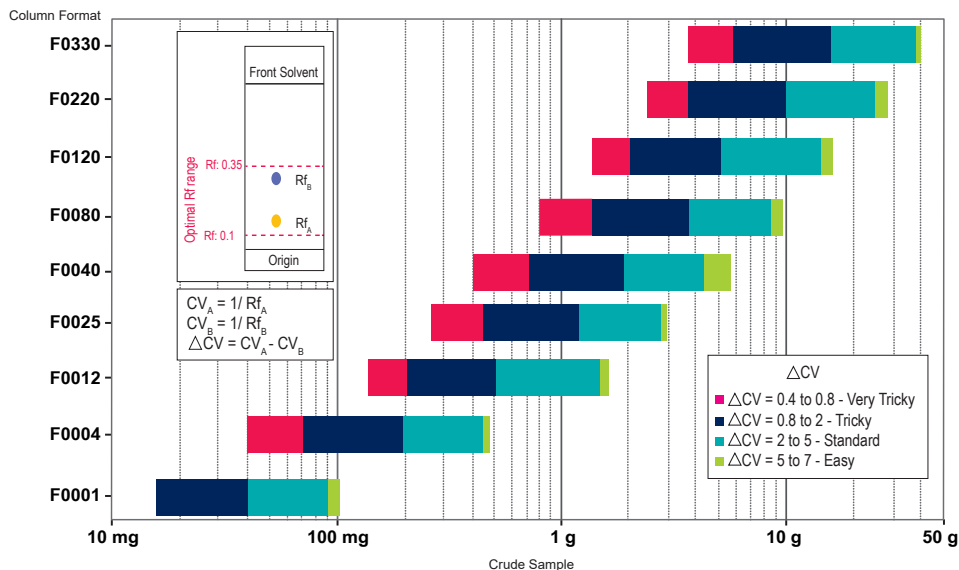
	F0004	5.2	7
CT-200D-I	F0012	21.3	7
CT-20ID	F0025	33.6	7
CT-20IA	F0040	54.0	7
CT-20IC	F0080	113.6	7
	F0120	165.9	7
	F0220	304.0	7

	F0004	5.2	4
	F0012	21.3	3
	F0025	33.6	3
PF-AC	F0040	54.0	3
	F0080	113.6	3
	F0120	165.9	2
	F0220	304.0	2
	F0330	456.7	2
	F0800	1174.6	2
	F1600	2333.4	2

	F0004	5.2	5
	F0012	21.3	5
	F0025	33.6	5
	F0040	54.0	5
PT-RPNH PP-RPNH	F0080	113.6	5
	F0120	165.9	5
	F0220	304.0	5
	F0330	456.7	5
	F0800	1174.6	5
	F1600	2333.4	5

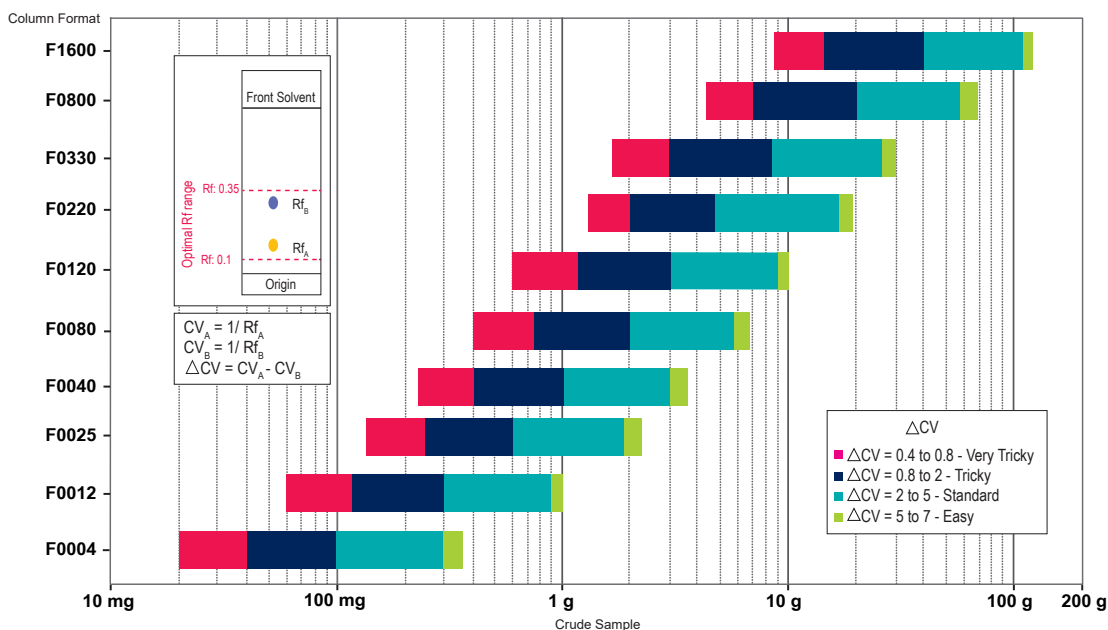


Loading Selection Guide for puriFlash® PF-15SIHP



Average values for compounds < 800 MW
These data depend on the conditions of elution and the products to be purified.

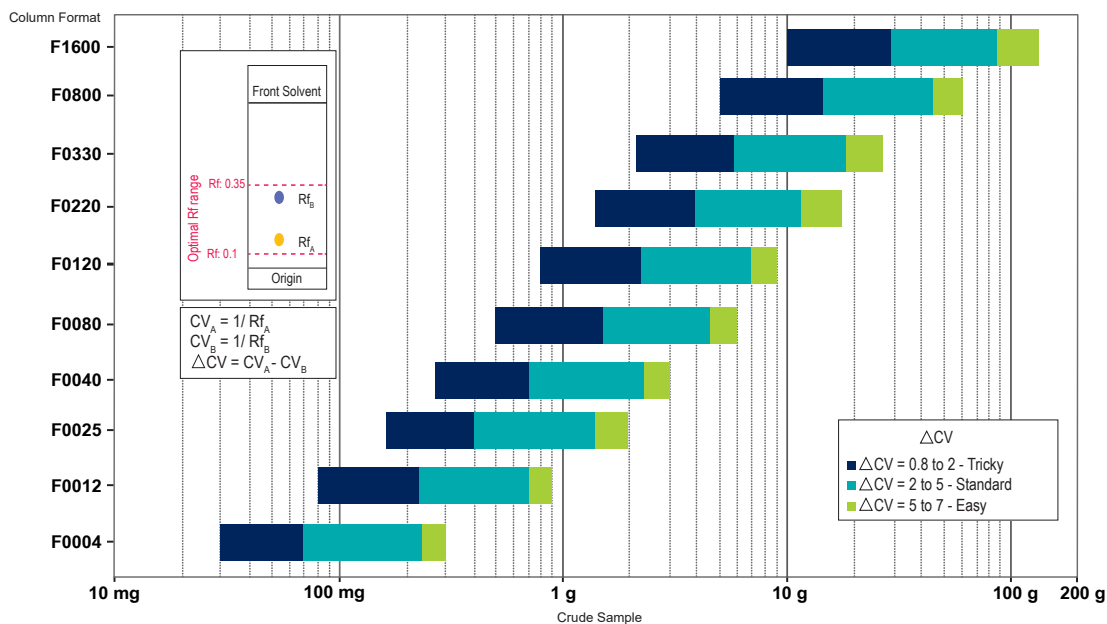
Loading Selection Guide for puriFlash® PF-30SIHP



Average values for compounds < 800 MW
These data depend on the conditions of elution and the products to be purified.

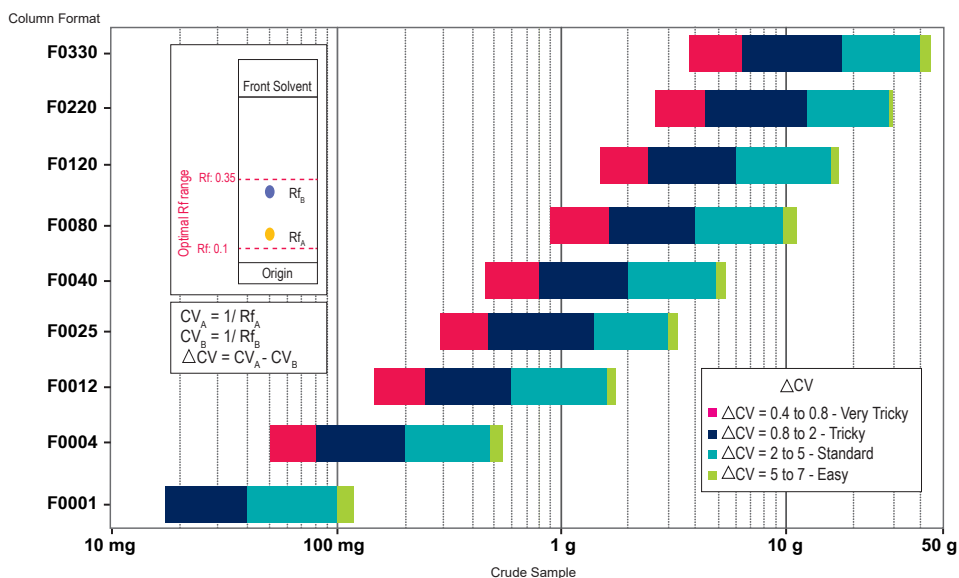


Loading Selection Guide for puriFlash® PF-50SIHP



Average values for compounds < 800 MW
These data depend on the conditions of elution and the products to be purified.

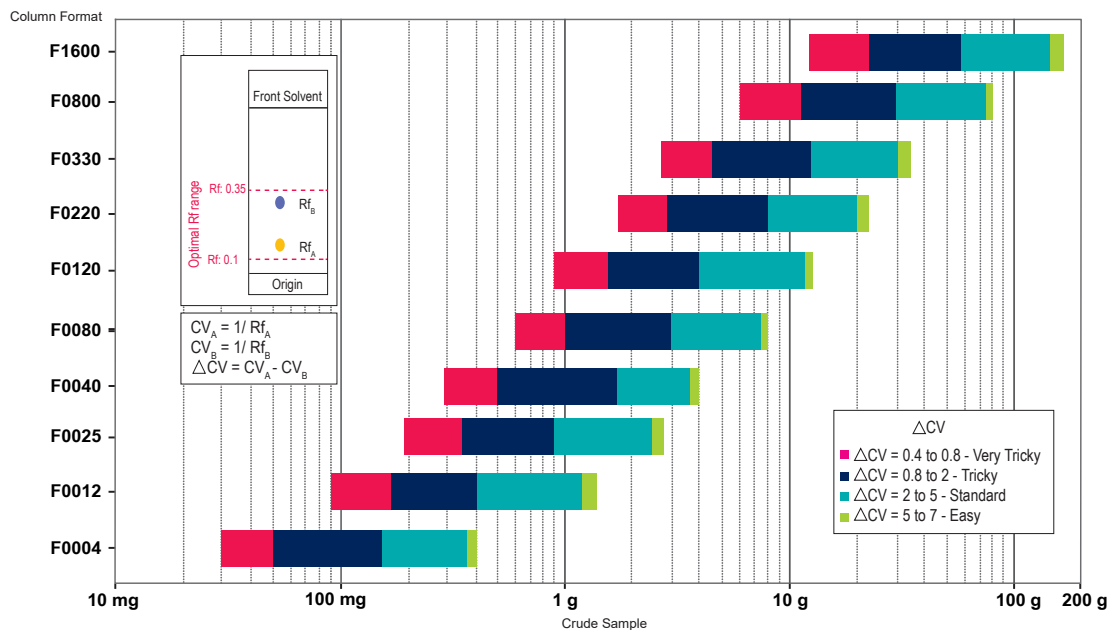
Loading Selection Guide for puriFlash® PF-15SIHC



Average values for compounds < 500 MW
These data depend on the conditions of elution and the products to be purified.



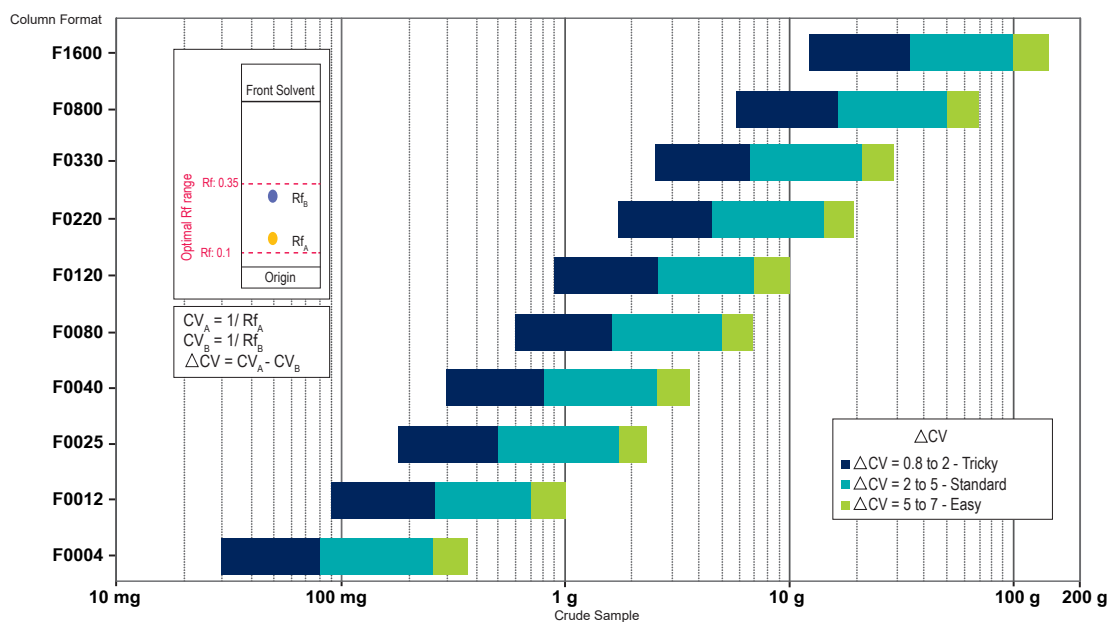
Loading Selection Guide for puriFlash® PF-25SIHC



Average values for compounds < 500 MW

These data depend on the conditions of elution and the products to be purified.

Loading Selection Guide for puriFlash® PF-50SIHC

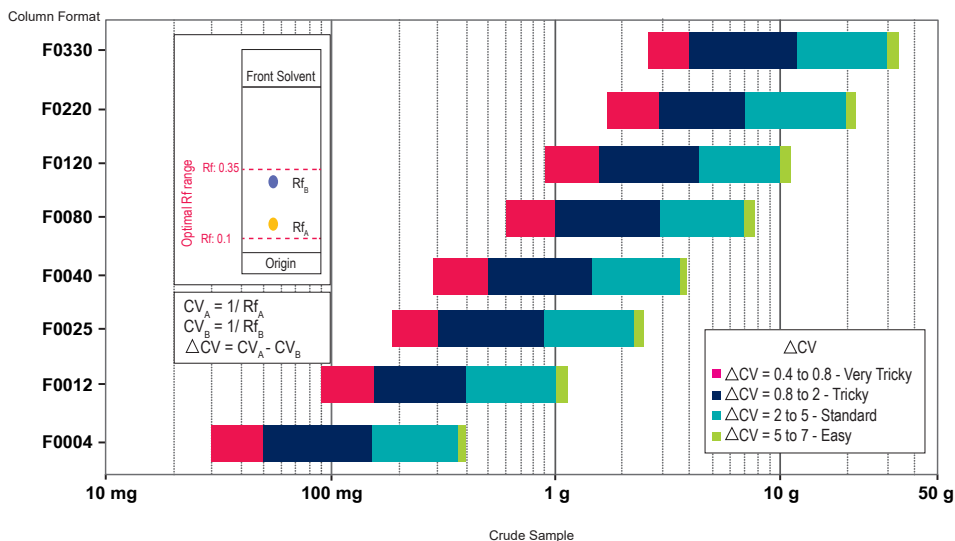


Average values for compounds < 500 MW

These data depend on the conditions of elution and the products to be purified.

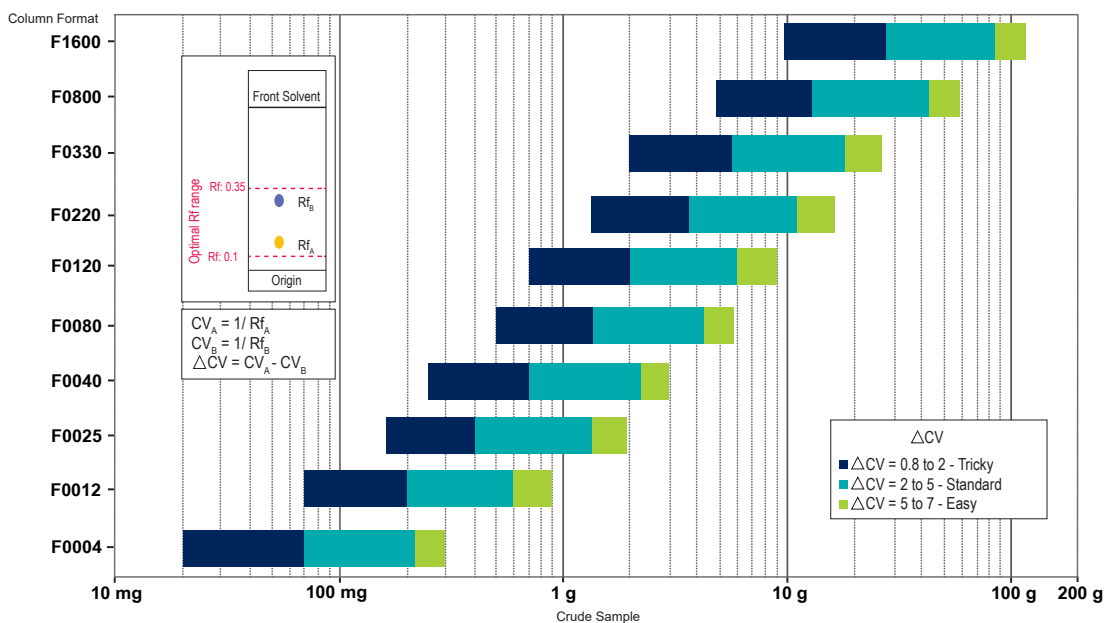


Loading Selection Guide for puriFlash® IR-20SI



Average values for compounds < 800 MW
These data depend on the conditions of elution and the products to be purified.

Loading Selection Guide for puriFlash® IR-50SI



Average values for compounds < 800 MW
These data depend on the conditions of elution and the products to be purified.



Loading capacity

The loading capacity depends on the ΔCV of the TLC plate or the resolution between 2 peaks obtained on the LC column. Higher the resolution and ΔCV are, the more important the load can be.

Loading: 0.1% to 0.3%

puriFlash® CT-20IA
puriFlash® CT-20IC
puriFlash® CT-20ID
puriFlash® CT-20OD-I

Loading: 0.1% to 0.7%

puriFlash® PT-C18AQ
puriFlash® PT-C8
puriFlash® PT-C4
puriFlash® PP-C18
puriFlash® PP-C4
puriFlash® Bio 200Å C18N, C18T, C18XS,
C8N, RPNH, RP
puriFlash® Bio 300Å C4-AQ, RPNH

Loading: 0.1% to 1.4%

puriFlash® C18HP
puriFlash® C18XS
puriFlash® C18AQ
puriFlash® RPAQ
puriFlash® C18T
puriFlash® CN
Uptisphere® CN
puriFlash® Diol
puriFlash® IR-C18
puriFlash® NH₂
puriFlash® NH₂HC
puriFlash® Bio 100Å C18N, C18XS,
C18T, C4AQ, RPNH
Uptisphere® C18-NEC
Uptisphere® Strategy™ C18HQ
Uptisphere® Strategy™ C18-3
Uptisphere® Strategy™ C18RP
Uptisphere® Strategy™ HIA
Uptisphere® Strategy™ HIT
Uptisphere® Strategy™ PHC4

Loading: 0.1% to 5%

puriFlash® Atoll X
puriFlash® P6

Loading: 0.1% to 10%

puriFlash® ALB
puriFlash® ALN
puriFlash® AgNO₃
puriFlash® AC

Loading capacity for bonded phases (RP & NP)

Loading capacity for bonded phases as a percentage of the adsorbent mass in the column						
		$\Delta k = 0.4$	$\Delta k = 0.8$	$\Delta k = 2$	$\Delta k = 5$	$\Delta k = 7$
15µm	60Å < pore size < 120Å	0,12%	0,20%	0,55%	1,30%	1,40%
	200Å < pore size < 300Å	0,06%	0,10%	0,25%	0,65%	0,70%
30µm	60Å < pore size < 120Å	0,07%	0,10%	0,30%	0,90%	1,10%
	200Å < pore size < 300Å	0,03%	0,06%	0,15%	0,45%	0,60%
50µm	60Å < pore size < 120Å	...	0,08%	0,20%	0,70%	0,90%
	200Å < pore size < 300Å	...	0,04%	0,10%	0,35%	0,50%

These values are given as an indication and may vary depending on the molecules and adsorbents used.



puriFlash® Care of Use, Cleaning & Storage

Storage before use:

Store the columns in a cool place, away from light and dust.
Do not remove the blue caps until the column is required for use.

First use:

puriFlash® columns fittings are Luer-type.
Attach the bottom of the column to a female Luer connector.
Attach the top of the column to a male Luer-lock connector.

Column conditioning:

This stage is essential to benefit of the full performance of the column.
It activates the silica & expels the air present in the column.
For optimum purification, this stage must be performed before the sample loading.
Rinse the column with 3 to 5 column volumes of solvent.
The solvent chosen is generally the solvent used at the start of the purification.



Conditioning with a strong eluent will lead to poor separation due to the high affinity of the solvent with the compounds to be purified.

Drying the column after the conditioning stage results in column performance loss.

Before use:

Store the column dry in a cool place & away from light & dust.

Stationary Phase	Activation	Cleaning	Storage
<p>puriFlash® C18HP, C18HQ, C18XS, C18, PhC4</p> <p>puriFlash® Bio C18N, C8N, C18T, 45RP, 50RPT, RPNH, C8, C4</p>	<p>Rinse with 20% MeOH - 80% water or 30% ACN - 70% water.</p> <p>A minimum of 3% of organic is required to keep the activation of the bonded phase.</p> <p>3 to 4 column volume are necessary.</p> <p>Once the column is activated never dry it.</p>	<p>After each run: Rinse with 3 to 4 column volumes of the strongest solvent (100%) to remove impurities.</p>	<p>After the final run: For a short term storage, flush the column with miscible solvents to reach at least a 20 to 25% of organic such as Acetonitrile, MeOH, Ethanol, Isopropanol...</p> <p>For a long term storage, flush the column with miscible solvents to reach at least a 50% of Acetonitrile (or MeOH) - 50% water or 100% of Isopropanol.</p> <p>For both, pass through 6 to 8 column volumes.</p>
<p>puriFlash® C18AQ</p> <p>puriFlash® RPAQ</p> <p>puriFlash® Bio C4AQ</p>	<p>Rinse with 20% MeOH - 80% water or 30% ACN - 70% water.</p> <p>Depending of the mobile phase chosen for the purification, rinse the column with 100% of water.</p> <p>3 to 4 column volume are necessary.</p> <p>Once the column is activated never dry it.</p>	<p>After each run: Rinse with 3 to 4 column volumes of the strongest solvent (100%) to remove impurities.</p>	<p>After the final run: For a short term storage, flush the column with miscible solvents to reach at least a 20 to 25% of organic such as Acetonitrile, MeOH, Ethanol, Isopropanol...</p> <p>For a long term storage, flush the column with miscible solvents to reach at least a 50% of Acetonitrile (or MeOH) - 50% water or 100% of Isopropanol.</p> <p>For both, pass through 6 to 8 column volumes.</p>
<p>puriFlash® Atoll X</p>	<p>Rinse with 20% MeOH - 80% water or 30% ACN - 70% water.</p> <p>A minimum of 5% of organic is required to keep the activation of the bonded phase.</p> <p>3 to 4 column volume are necessary.</p> <p>Once the column is activated never dry it.</p>	<p>After each run: Rinse with 3 to 4 column volumes of the strongest solvent (100%) to remove impurities.</p>	<p>After the final run: For a short term storage, flush the column with miscible solvents to reach at least a 20 to 25% of organic such as Acetonitrile, MeOH, Ethanol, Isopropanol...</p> <p>For a long term storage, flush the column with miscible solvents to reach at least a 50% of Acetonitrile (or MeOH) - 50% water or 100% of Isopropanol.</p> <p>For both, pass through 6 to 8 column volumes.</p>



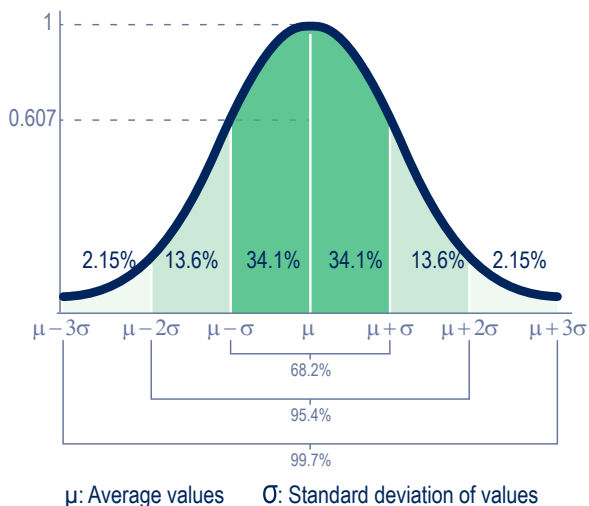
Before use:

Store the column dry in a cool place & away from light & dust.

Stationary Phase	Activation	Cleaning	Storage
puriFlash® CN puriFlash® NH2 puriFlash® Diol	<p>If the column will be used under NP mode: Rinse with 100% Heptane. 3 to 4 column volume are necessary. Once the column is activated never dry it. Do not leave the column under Heptane or Hexane for more than a day.</p> <p>If the column will be used under RP mode: Rinse with 20% MeOH - 80% water or 30% ACN - 70% water. A minimum of 5% of organic is required to keep the activation of the bonded phase. 3 to 4 column volume are necessary. Once the column is activated never dry it.</p>	<p>After each run: Rinse with 3 to 4 column volumes of the strongest solvent (100%) to remove impurities.</p>	<p>After the final run: For a short term storage, flush the column with miscible solvents to reach at least a 20 to 25% of Ethanol or Isopropanol... For a long term storage, flush the column with miscible solvents to reach at least a 50% of Ethanol - 50% water or 100% of Isopropanol. For both, pass through 6 to 8 column volumes.</p>
puriFlash® SCX puriFlash® MM1	<p>Rinse with 20% MeOH - 80% water or 30% ACN - 70% water. 6 to 8 column volume are necessary. Once the column is activated never dry it.</p>	<p>After each run: Rinse with 6 to 8 column volumes of the strongest solvent (100%) to remove impurities.</p>	<p>After the final run: For a short term storage, flush the column with miscible solvents to reach at least a 20 to 25% of Ethanol or Isopropanol plus 0.5% of Sodium azide. For a long term storage, flush the column with miscible solvents to reach 1M acetic acid in MeOH, pass through 8 to 10 column volumes. then reach at least a 50% of Ethanol - 50% water plus 0.5% of Sodium azide or 100% of Isopropanol plus 0.5% of Sodium azide.</p>
puriFlash® SAX	<p>Rinse with 20% MeOH - 80% water or 30% ACN - 70% water. 6 to 8 column volume are necessary. Once the column is activated never dry it.</p>	<p>After each run: Rinse with 6 to 8 column volumes of the strongest solvent (100%) to remove impurities.</p>	<p>After the final run: For a short term storage, flush the column with miscible solvents to reach at least a 20 to 25% of Ethanol or Isopropanol plus 0.5% of Sodium azide. For a long term storage, flush the column with miscible solvents to reach 5% NH4OH in MeOH, pass through 8 to 10 column volumes. then reach at least a 50% of Ethanol - 50% water plus 0.5% of Sodium azide or 100% of Isopropanol plus 0.5% of Sodium azide.</p>
HILIC - HIA	<p>Rinse with 70% ACN - 30% water. 3 to 4 column volume are necessary. Once the column is activated never dry it.</p>	<p>After each run: Rinse with 3 to 4 column volumes of the strongest solvent (100%) to remove impurities.</p>	<p>After the final run: For a short term storage, flush the column with miscible solvents to reach at a 70% of Acetonitrile. For a long term storage, flush the column with miscible solvents to reach at least a 100% of Acetonitrile. For both, pass through 6 to 8 column volumes. Avoid Ethanol, Methanol, Acetone, Isopropanol.</p>
CT-20IA CT-20IC CT-20ID CT-OD-I	For more details, please refer to www.chiral.fr		<p>Never storage under Hexane, Cyclohexane, Heptane For a long term storage, flush the column with miscible solvents to reach 100% of EtOH Pass through 6 to 8 column volumes of 100 % EtOH.</p>
P6	<p>Active the stationary phase with the starting eluent condition. Once the column is activated never dry it.</p>	<p>After each run: Rinse with 3 to 4 column volumes of the strongest solvent (100%) to remove impurities.</p>	<p>After the final run: Flush the column with 100 % of Isopropanol. Avoid Dichloromethane, DMF.</p>



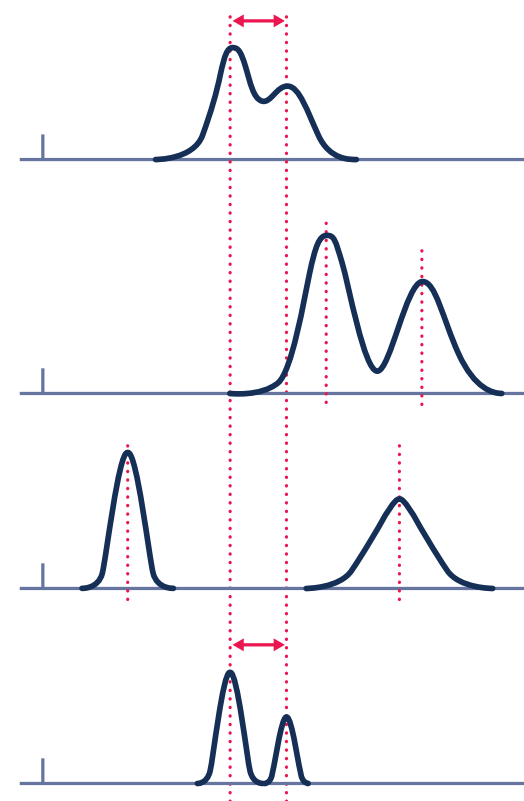
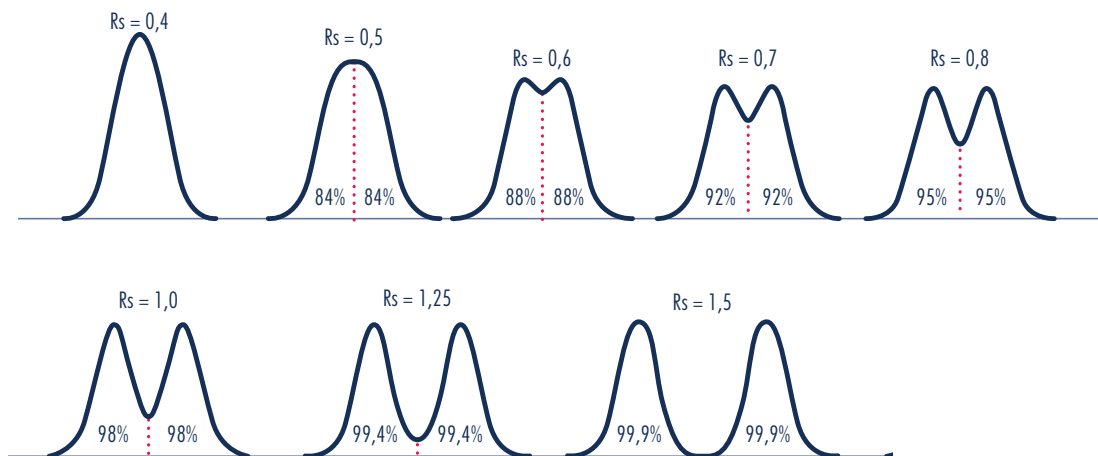
Gaussian Representation of a Peak



NOTES



Peaks Shape according to R_s & its relative heights



High particle diameter and incomplete separation

Improvement by:

- increasing retention, decreasing the eluting force
- increasing the quantity of stationary phase (column size)
- changing stationary phase

- modifying the selectivity (gradient, proportion of solvent, choice of other solvents)

- increasing the efficiency (number of plates) with smaller particles size

↔ Separation estimated by selectivity α (LC) or ΔR_f (TLC)



Usual Buffers in HPLC

pKa	Buffer	Buffer range	LC/MS compatibility
0.3	TFA (0.1%)	1.8	Yes
2.1 (pK1)	Phosphate	1.1 - 3.1	No
3.1 (pK1)	Citrate	2.1 - 4.1	No
3.8	Ammonium formate	2.8 - 4.8	Yes
3.8	Formic acid (0.1%)	2.7	Yes
4.7 (pK2)	Citrate	3.7 - 5.7	No
4.8	Ammonium acetate	3.8 - 5.8	Yes
4.8	Acetic acid (0.1%)	3.3	Yes
6.4 (pK3)	Citrate	4.4 - 6.4	No
7.2 (pK2)	Phosphate	6.2 - 8.2	No
7.6	Ammonium bicarbonate	6.6 - 11.3	Yes
8.3	Tris	7.3 - 9.3	No
9.2	Borate	8.2 - 10.2	No
9.2	Ammonia 25% (0.1%)	8.8	Yes
10.7	Triethylamine acetate	9.7 - 11.7	Yes
12.3 (pK3)	Phosphate	11.3 - 13.3	No

Concentrations

%	10 ^x	ppm	ppb	ppt	mg/mL or µg/µL
1	10 ⁻²	10 000	10 000 000	10 000 000 000	10
0.1	1x10 ⁻³	1 000	1 000 000	1 000 000 000	1
0.01	1x10 ⁻⁴	100	100 000	100 000 000	0.1
0.001	1x10 ⁻⁵	10	10 000	10 000 000	0.02
0.0001	1x10 ⁻⁶	1	1 000	1 000 000	0.001
0.00001	1x10 ⁻⁷	0,1	100	100 000	0.0001
0.000001	1x10 ⁻⁸	0,01	10	10 000	0.00001
0.0000001	1x10 ⁻⁹	0,001	1	1 000	0.000001