





SepaFlash HILIC Cartridges

A complementary technique to reversed-phase LC for fast and efficient purification of hydrophilic samples

HILIC can be regarded as a complementary technique to reversed-phase HPLC, owing to the differences in elution order of solutes and its good retention of hydrophilic species, which are difficult to retain in reversed-phase LC. In addition, it has the following advantages:



- Higher flow rates can be used due to the low viscosity of mobile phases of high organic content, thus relatively greater efficiencies can be obtained compared with reversed-phase LC.
- It might be possible to inject extracts directly from C18 solid-phase extraction (SPE) columns, from which solutes usually are eluted with high organic content. Use of an LC mechanism that is different from that of the sample preparation also introduces a degree of orthogonality into the overall procedure, contributing to a higher peak resolution.
- Avoid of using ion pair reagents, which is especially beneficial for preparative LC procedure due to ease of handling with solvents.
- Enhanced detection sensitivity when used in conjunction with mass spectrometry (MS) or evaporated light scattering detector (ELSD). A sensitivity increase of as much as three orders of magnitude was demonstrated for the analysis of some hydrophilic samples in comparison with reversed-phase LC–MS.

Sorbent	Structure	characteristics
Amine		Irregular, 40-63 μm . Endcapping: Yes. Amino content: 1.3 mmol/g
Diol		Spherical, 20-45 μm . Endcapping: Yes. Carbon content: 5.0%
Cyano		Spherical, 20-45 μm . Endcapping: Yes. Carbon content: 5.5%
ARG		Spherical, 20-45 μm . Endcapping: Yes. Carbon content: 8.0%

Pre-packed Media Options

NEW! High-efficiency spherical ARG, 20-45 μm , 100 Å

(carbon content 8%, surface area 320 m^2/g , loading capacity 0.1-2%)

Item Number	Column Size	Sample Size	Units/Box	Flow Rate (mL/min)	Cartridge Length (mm)	Cartridge ID (mm)	Max. Pressure (psi/bar)
SW-5622-004-SP	5.4 g	5.4 mg-108 mg	2	5-15	113.8	12.4	400/27.5
SW-5622-012-SP	20 g	20 mg-0.40 g	1	10-25	134.8	21.4	400/27.5
SW-5622-025-SP	33 g	33 mg-0.66 g	1	10-25	184	21.4	400/27.5
SW-5622-040-SP	48 g	48 mg-0.96 g	1	15-30	184.4	26.7	400/27.5
SW-5622-080-SP	105 g	105 mg-2.1 g	1	20-50	257.4	31.2	350/24.0
SW-5622-120-SP	155 g	155 mg-3.1 g	1	30-60	261.5	38.6	300/20.7
SW-5622-220-SP	270 g	300 mg-6.0 g	1	40-80	223.5	61.4	300/20.7
SW-5622-330-SP	420 g	420 mg-8.4 g	1	40-80	280.2	61.4	250/17.2

- Compatible with all flash chromatography systems, for example ISCO, Biotage, Yamazen, etc.

UltraPure irregular NH₂, 40-63 µm, 60 Å(amino content 1.3 mmol/g, end-capping, surface area 500 m²/g, loading capacity 0.1–2%)

Item Number	Column Size	Sample Size	Units/Box	Flow Rate (mL/min)	Cartridge Length (mm)	Cartridge ID (mm)	Max. Pressure (psi/bar)
SW-5501-004-IR	5.9 g	5.9 mg–118 mg	2	10–20	113.8	12.4	400/27.5
SW-5501-012-IR	23 g	23 mg–0.46 g	1	15–30	134.8	21.4	400/27.5
SW-5501-025-IR	38 g	38 mg–0.76 g	1	15–30	184.0	21.4	400/27.5
SW-5501-040-IR	55 g	55 mg–1.1 g	1	20–40	184.4	26.7	400/27.5
SW-5501-080-IR	122 g	122 mg–2.5 g	1	30–60	257.4	31.2	350/24.0
SW-5501-120-IR	180 g	180 mg–3.6 g	1	40–80	261.5	38.6	300/20.7
SW-5501-220-IR	340 g	340 mg–6.8 g	1	50–100	223.5	61.4	300/20.7
SW-5501-330-IR	475 g	475 mg–9.5 g	1	50–100	280.2	61.4	250/17.2

- Compatible with all flash chromatography systems, for example ISCO, Biotage, Yamazen, etc.

High-efficiency spherical Diol, 20-45 µm, 100 Å(carbon content 5%, end-capping, surface area 320 m²/g, loading capacity 0.1–2%)

Item Number	Column Size	Sample Size	Units/Box	Flow Rate (mL/min)	Cartridge Length (mm)	Cartridge ID (mm)	Max. Pressure (psi/bar)
SW-5922-004-SP	5.4 g	5.4 mg–108 mg	2	5–15	113.8	12.4	400/27.5
SW-5922-012-SP	20 g	20 mg–0.40 g	1	10–25	134.8	21.4	400/27.5
SW-5922-025-SP	33 g	33 mg–0.66 g	1	10–25	184.0	21.4	400/27.5
SW-5922-040-SP	48 g	48 mg–0.96 g	1	15–30	184.4	26.7	400/27.5
SW-5922-080-SP	105 g	105 mg–2.1 g	1	20–50	257.4	31.2	350/24.0
SW-5922-120-SP	155 g	155 mg–3.1 g	1	30–60	261.5	38.6	300/20.7
SW-5922-220-SP	300 g	300 mg–6.0 g	1	40–80	223.5	61.4	300/20.7
SW-5922-330-SP	420 g	420 mg–8.4 g	1	40–80	280.2	61.4	250/17.2

- Compatible with all flash chromatography systems, for example ISCO, Biotage, Yamazen, etc.

High-efficiency spherical CN, 20-45 µm, 100 Å(carbon content 5.5%, end-capping, surface area 320 m²/g, loading capacity 0.1–2%)

Item Number	Column Size	Sample Size	Units/Box	Flow Rate (mL/min)	Cartridge Length (mm)	Cartridge ID (mm)	Max. Pressure (psi/bar)
SW-5322-004-SP	5.4 g	5.4 mg–108 mg	2	5–15	113.8	12.4	400/27.5
SW-5322-012-SP	20 g	20 mg–0.40 g	1	10–25	134.8	21.4	400/27.5
SW-5322-025-SP	33 g	33 mg–0.66 g	1	10–25	184.0	21.4	400/27.5
SW-5322-040-SP	48 g	48 mg–0.96 g	1	15–30	184.4	26.7	400/27.5
SW-5322-080-SP	105 g	105 mg–2.1 g	1	20–50	257.4	31.2	350/24.0
SW-5322-120-SP	155 g	155 mg–3.1 g	1	30–60	261.5	38.6	300/20.7
SW-5322-220-SP	300 g	300 mg–6.0 g	1	40–80	223.5	61.4	300/20.7
SW-5322-330-SP	420 g	420 mg–8.4 g	1	40–80	280.2	61.4	250/17.2

- Compatible with all flash chromatography systems, for example ISCO, Biotage, Yamazen, etc.

Application I. The Purification of Hydrophilic Compounds

In HILIC, hydrophilic, polar, and charged compounds are retained preferentially compared with hydrophobic neutral compounds — the opposite of reversed-phase LC. In this application, two strong polar compounds (Figure 1) were used as the sample to evaluate the separation efficiency of the SepaFlash HILIC ARG cartridge. The results (Figure 2 and Figure 3) showed that for the separation and purification of the sample of strong polarity, the SepaFlash HILIC ARG cartridge was a good choice as compared with SepaFlash C18 cartridge which was run in reversed-phase.

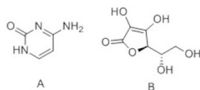


Figure 1. The structural formula of two strong polar compounds. (A: cytosine. B: vitamin C.)

Instrument	A preparative flash chromatography system			
Flash cartridge	25g SepaFlash® HILIC ARG cartridge (Order number: SW-5622-025-SP) 25g SepaFlash® C18 cartridge (Order number: SW-5222-025-SP)			
Sample loading	10 mg of the mixture			
Wavelength	220 nm, 254 nm			
Mobile phase	Solvent A: 200 mM ammonium acetate (pH 9.0) in water Solvent B: acetonitrile			
Flow rate	15 ml/min			
Gradient	Settings for C18 cartridge		Settings for HILIC cartridge	
	Solvent B (%)	Time (min)	Solvent B (%)	Time (min)
	5	0	95	0
	5	3	95	5
	5	5	80	10
	98	30	70	13
	-	-	70	30

Table 1. The experimental parameters.

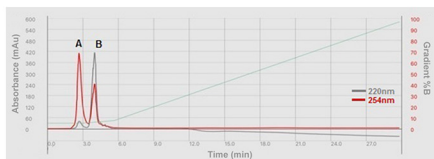


Figure 3. Separation of the sample using a SepaFlash® C18 cartridge in reversed-phase.

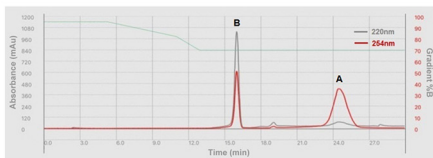


Figure 4. Separation of the sample using a SepaFlash® HILIC ARG cartridge.

Application II. The Purification of Polar Synthetic Intermediates

The compounds of strong polarity or hydrophilicity could barely be retained in reversed-phase chromatography. In the meantime they could hardly be dissolved in the mobile phase of normal phase chromatography. Therefore HILIC cartridges were employed for the separation and purification of these samples with specific characteristics. In this application, a 40g SepaFlash HILIC ARG cartridge was utilized to purify strong hydrophilic samples (Figure 5). As a comparison, a 40g SepaFlash C18 cartridge was also used in a parallel experiment. The results showed that SepaFlash HILIC flash cartridge is a good choice for the separation and purification of samples with strong polarity or hydrophilicity.

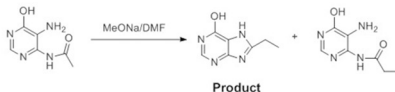


Figure 5. The chemical structure of the sample mixture.

Instrument	A flash preparative chromatography system			
Flash cartridge	40g SepaFlash® HILIC ARG cartridge (Order number: SW-5622-040-SP) 40g SepaFlash® C18 cartridge (Order number: SW-5222-040-SP)			
Wavelength	254 nm (detection), 280 nm (monitoring)			
Mobile phase	Solvent A: water		Solvent B: acetonitrile	
Flow rate	30 ml/min			
Loading capacity	100 mg of the mixture			
Gradient	Settings for C18 cartridge		Settings for HILIC cartridge	
	Solvent B (%)	Time (min)	Solvent B (%)	Time (min)
	5	0	95	0
	5	3	95	15
	22	9	50	17
	22	12	/	/

Table 1. The experimental parameters.

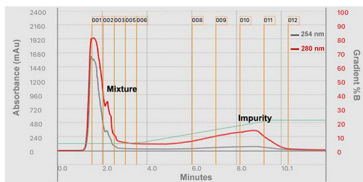


Figure 3. The chromatogram of the sample mixture in C18 cartridge.

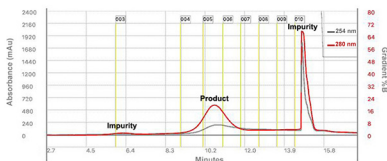


Figure 4. The chromatogram of the sample mixture in HILIC ARG cartridge.

Notices for Using the HILIC Cartridges

The equilibrium for HILIC cartridges

- I. Use 10 CVs (column volume) of ACN-water (v:v = 50:50) to equilibrate the new HILIC cartridge.
- II. Before sample loading, use 5 CVs of initiate mobile phase to equilibrate the cartridge.

Notices for sample loading in HILIC separation mode

- I. If possible, use 100% ACN as the solvent to dissolve the sample. Avoid using pure water or DMSO as the sample solvent since they will result in peak shape deterioration.
- II. The recommended solvent for sample dissolving is weak HILIC solvents such as ACN, MeOH, isopropanol, etc.
- III. The most popular sample solvent is ACN-MeOH (v:v = 75:25). This solvent system achieves a good balance between sample solubility and peak shape.
- IV. Before sample loading, replace the water or DMSO with ACN in the sample by reversed SPE (solid phase extraction) method. If such operation is impossible, use organic solvents to dilute the water or DMSO in the sample.

Notices for the mobile phase used in HILIC separation mode

- I. Always keep at least 5% polar solvent (such as 5% water phase buffer, 5% MeOH or 3% MeOH / 2% water phase buffer, etc.) in the mobile phase since this will ensure the silica gels packed in the HILIC cartridge are always wetted by water.
- II. Always keep the percentage of the organic solvent in the mobile phase not lower than 40% when running isocratic or gradient elution. For example, for ACN-water system, keep the percentage of ACN in mobile phase not lower than 40%.
- III. Do not use phosphate buffer system since phosphate may precipitate from the buffer in HILIC separation mode. Use phosphoric acid instead.

- IV. Comparing with formic acid or acetic acid, the buffer system such as ammonium formate or ammonium acetate has better reproducibility in results.
- V. To achieve best peak shape, always keep the concentration of the buffer system as 10mM in the mobile phase or gradient profile.

Other suggestion about using HILIC separation mode

- I. As a start, a gradient profile of 95% ACN down to 50% ACN in the mobile phase is recommended. If the sample has poor retention, use ACN/MeOH/water (v:v = 95:3:2) buffer system as the mobile phase for isocratic elution.
- II. To increase the retention of the polar compounds, replace the water in the mobile phase with MeOH, acetone or isopropanol.
- III. Please make sure that the flushing solvent has the same high percentage of organic phase as the mobile phase. Otherwise the peak shape would be deteriorated.

Cleaning and regeneration of the HILIC cartridge

When separating samples by HILIC cartridges, if chromatographic resolution is deteriorated, or peak shape broadens, or column pressure goes higher or other abnormal situation occurs, it is probably due to some dirty stuff of strong retention to the stationary phase of the cartridge which changes the column capacity so that the interaction between the sample and the stationary phase is compromised. In this circumstance, the cartridge should be thoroughly cleaned and maintained.

- I. **Cleaning:** To remove polar contaminant, flush the HILIC cartridge with 50% ACN in water at low flow rate for 20 column volumes (CV). In case of no effect, a solvent consisting of 5% ACN in water should be used for washing the cartridge.
- II. **Preservation:** The HILIC cartridge should be preserved in the solvent consisting of 95% ACN in water. NEVER preserve the cartridge in the mobile phase with buffer salts. If mobile phase with buffer salts was used, deionized water should be employed to wash the cartridge for 10 CVs and then replaced by 95% ACN in water for cartridge preservation.

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