Agela Technologies

Claricep™

Claricep Columns Care Use and Manual

Thank you for purchasing Claricep flash columns. Below are recommended instructions for the care and use of your Claricep flash column.

General Information

This user's manual is applicable only for the use of Claricep flash irregular and spherical silica columns manufactured by Bonna-Agela Technologies. Please read this instruction manual carefully before use. Claricep flash products use homogeneous packing which results in no channeling (no peak tailing), high loading capacity (high surface area > 350m^2/g), and offers a solution of direct transfer from TLC or HPLC.

Compatibility

Claricep Flash Cartridges are compatible with a wide variety of instruments on the market: Agela, Analogix, Armen Instrument, Biotage, Büchi, ECOM, GE ÄKTA, Gilson, Grace, Interchim, Santai Technologies, Teledyne Isco, Yamazen, etc.

Installation

1. Open the package and remove both end plugs attached to the flash column and place them in the box for future use.
2. Align the flash column to a proper height within the flash system and connect the fittings. Each Claricep flash column has standard fittings which facilitate easy connections to intermediate pressure instruments from various vendors.
3. For storage, place the cartridges in a dry place, away from light and dust, and at room temperature. Do not remove end caps for storage.

Column Condition Recommendations

Steps	Reversed Phase Mode	Normal Phase Mode	HILIC Phase Mode
Pre-conditioning	Flush the column 3x Column Volume (3CV) with 90% organic solvent	Flush the column 3CV with 90% weak organic solvent	Flush the column 5CV with >95% organic solvent.
Equilibration	Minimum 5CV with the starting conditions	Minimum 3CV with the starting conditions	Minimum 10CV with the starting conditions
Post-Run Cleaning	• Rinse 5CV of 95:5 Water/Organic (for buffer or additive removal if any) and then 5CV 95:5 Organic/Water • For hydrophobic or oily materials, try flushing with 5CV of IPA, after the column has been flushed with Acetonitrile. When using IPA, ensure use of a low flow to prevent higher backpressures due to higher solvent viscosity • For very hydrophobic materials, try flushing 5CV of 50:50 IPA/THF	• Rinse with 5CV of 100 % of the most polar (stronger) solvent in the elution mixture • If needed, rinse with 5CV of 100% IPA at low flow rate • NB: "single use" recommended for columns packed with silica	• Rinse with at least 10CV of 95:5 Water/ Acetonitrile • Repeat with 95:5 100 mM Ammonium Acetate (pH 5.8)/Acetonitrile. • Then finish cleaning by flushing the column with 95:5 Water/Acetonitrile
Storage Conditions	• Short-term storage (< 1 week): 3CV of > 60 % organic solvent • Long-term storage (> 1 week): 3CV of > 80 % organic solvent	• 100% IPA for bonded Phases	• Short-term storage (< 1 week): 5CV of > 70% Acetonitrile solvent • Long-term storage(> 1 week): 5CV of 100% acetonitrile solvent
Mobile Phase	• Use only high purity chemicals and reagents. • Check for miscibility/solubility when changing solvents and buffers. • Ensure sample (and matrix) are completely soluble/miscible with mobile phase. • Immiscible solvents or salt precipitation can permanently damage the column.		
pH Range	• Recommended: 1.5-8.5 • Volatile buffers and pH modifiers are strongly recommended because they simplify compound recovery post purification. • pH < 1.5 will strip the bonded phase • pH > 8.5 will dissolve the silica • Avoid immiscible solvents and buffers; trace impurities		

Sample Loading

Solid Sample Loading

Option 1 Use an i-Series Claricep flash screw-on column. This is a new feature of Claricep columns that allows the user to open the cap of the column and load solid sample directly on the column. See Figure 1.

Option 2 Use an Empty Flash Column (EFC). Insert the EFC in the inlet port of the flash cartridge as demonstrated in Figure 2. EFC should have a luer lock end-fitting to prevent any backpressure and safety concern. Make sure to select s-Series of the EFC, part number “FCHXXX-S”.

Liquid Sample Loading

Inject the liquid sample directly into the flash column using the sample injector. In case of viscous sample injection, we recommend using c-Series. These columns feature a screw-on lid offering more convenience to load directly into the flash column head as demonstrated in Figure 2.

Figure 1: Screw-on Flash Columns

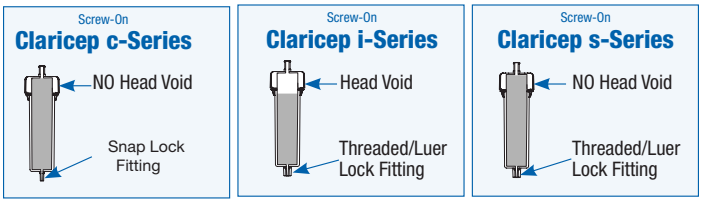
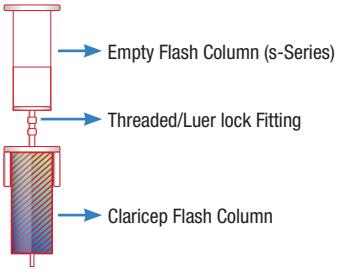


Figure 2: Empty Flash Column with Regular Flash Column



Flash Column Parameters (Reverse Phase and Normal Phase)

Specifications	4 g	12 g	20 g	40 g	80 g	120 g	220 g	330 g	800 g	1500 g	3000 g	5000 g
Sample Load1*	0.01-0.02g	0.03-0.06g	0.05-0.1g	0.1-0.2g	0.2-0.4g	0.3-0.6g	0.5-1.0g	0.75-1.5g	2-4g	3.75-7g	7.5-15g	15-30g
Sample Load2*	0.02-0.08g	0.06-0.24g	0.1-0.4g	0.2-0.8g	0.4-1.6g	0.6-2.4g	1.0-4.0g	1.5-6.0g	4-16g	7-28g	15-60g	30-120g
Sample Load3*	0.08-0.4g	0.24-1.2g	0.4-2.0g	0.8g-4.0g	1.6-8.0g	2.4-12.0g	4.0-22.0g	6.0-33.0g	16-80g	28-150g	60-300g	120-500g
Column Volume (mL)	8	17	34	64	115	200	290	380	1080	2000	4000	8000
Mini Flow Rate (mL/min)	5	8	10	20	25	35	45	50	150	240	350	500
Max Flow Rate mL/min)	18	20	25	40	50	80	90	100	200	300	450	650
Recommended Flow Rate (mL/min)	10	15	18	30	40	60	70	75	180	270	400	550
Pressure Max	180 PSI / 12 BAR										120 PSI / 8 BAR	
Length (cm)	7.0	9.0	11.0	14.0	21.0	23.5	15.7	23.5	34.8	37.0	47.0	60.0
Diameter (cm)	1.5	2.1	2.6	3.1	3.2	4.1	5.7	5.7	8.0	9.5	11.9	14.0
Ratio of Length/Diameter	4.7	4.3	4.2	4.5	6.6	5.7	2.8	4.1	4.4	3.9	3.9	4.3
Equilibration Volume (CV)	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	2.0	2.0	2.0	2.0
Equilibration Time (min)	2.4	3.4	5.7	6.4	8.6	10.0	8.3	10.1	12.0	14.8	20.0	29.1

* Note: Sample loading volume refering to ΔCV=1/Rf1-1/Rf2 - CV Range recommended - 2.5 to 10 CV (Rf range 0.1 to 0.4)
Sample Load1 - ΔCV= 1 to 2 ; Sample Load2 - ΔCV= 3 to 5, Sample Load3 - ΔCV = 6 to 7.5
The chart is based on using IRR 40-60 µm silica columns under normal phase conditions

Solvent Miscibility Table

Solvent	Polarity Index	Refractive Index @ 20°C	UV(nm) Cutoff @ 1AU	Boiling Point (C°)	Viscosity (Poise)	Solubility in Water (% w/w)
Acetic Acid	6.2	1.372	230	118	1.26	100
Acetone	5.1	1.359	330	56	0.32	100
Acetonitrile	5.8	1.344	190	82	0.37	100
Benzene	2.7	1.501	280	80	0.65	0.18
n-Butanol	4.0	1.394	254	125	0.73	0.43
Butyl Acetate	3.9	1.399	215	118	2.98	7.81
Carbon Tetrachloride	1.6	1.466	263	77	0.97	0.08
Chloroform	4.1	1.446	245	61	0.57	0.815
Cyclohexane	0.2	1.426	200	81	1.00	0.01
1,2-Dichloroethane[1]	3.5	1.444	225	84	0.79	0.81
Dichloromethane[2]	3.1	1.424	235	41	0.44	1.6
Dimethylformamide	6.4	1.431	268	155	0.92	100
Dimethyl Sulfoxide[3]	7.2	1.478	268	189	2.00	100
Dioxane	4.8	1.422	215	101	1.54	100
Ethanol	5.2	1.360	210	78	1.20	100
Ethyl Acetate	4.4	1.372	260	77	0.45	8.7
Di-Ethyl Ether	2.8	1.353	220	35	0.32	6.89
Heptane	0.0	1.387	200	98	0.39	0.0003
Hexane	0.0	1.375	200	69	0.33	0.001
Methanol	5.1	1.329	205	65	0.60	100
Methyl-tButyl Ether[4]	2.5	1.369	210	55	0.27	4.8
Methyl Ethyl Ketone[5]	4.7	1.379	329	80	0.45	24
Pentane	1.0	1.358	200	36	0.23	0.004
n-Propanol	4.0	1.384	210	97	2.27	100
Iso-Propanol[6]	3.9	1.377	210	82	2.30	100
Di-Iso-Propyl Ether	2.2	1.368	220	68	0.37	
Tetrahydrofuran	4.0	1.407	215	65	0.55	100
Toluene	2.4	1.496	285	111	0.59	0.051
Trichloroethylene	1.0	1.477	273	87	0.57	0.11
Water	9.0	1.333	200	100	1.00	100
Xylene	2.5	1.500	290	139	0.61	0.018

Immiscible

Miscible

Immiscible means that in some proportions two phases will be produced

Synonym Table

[1] Ethylene Chloride
[2] Methylene Chloride
[3] Methyl Sulfoxide
[4] tert-Butyl Methyl Ether
[5] 2-Butanone
[6] 2-Propanol

Solvent Polarity Chart

Relative Polarity	Compound Formula	Group	Representative Solvent Compounds
Nonpolar	R - H	Alkanes	Petroleum eithers, ligroin, hexanes
Increasing Polarity ↓	Ar - H	Aromatics	Toluene, benzene
	R - O - R	Ethers	Diethyl ether
	R - X	Alky halides	Tetrachloromethane, chloroform
	R - COOR	Esters	Ethyl acetate
	R - CO - R	Aldehydes and ketones	Acetone, methyl ethyl ketone
	R - NH_2	Amines	Pyridine, triethylamine
	R - OH	Alcohols	Methanol, ethanol, isopropanol, butanol
	R - $COHN_2$	Amides	Dimethylformamide
	R - COOH	Carboxylic acids	Ethanoic acid
Polar	H - OH	Water	Water

Additional Tips for Claricep Flash Cartridges

- Do not use 100% water with general reversed phase columns as it can promote phase collapse; a minimum of 2-3% Organic is usually required to keep the phase wetted. Only the AQ C18 phase is 100% aqueous stable.
- Once columns have been wetted, make sure to never dry out the columns. Store the columns in solvent with end caps in place. This will induce channeling due to expansion and contraction of the media phase.
- Always pre-condition columns before initial use, especially for reversed phase columns.
- After use, cap the flash column with plugs to prevent evaporation of mobile phase.
- Column conditioning is crucial to achieve the maximum performance of the column. It activates the silica and removes the air from the cartridge and prepares the stationary phase to retain the sample.
- Regular Claricep flash columns should not be disassembled, only “Screw-on” Flash columns have a cap that can be opened.

Guidance for sample loading in HILIC mode:

- If possible, use 100% acetonitrile 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. The recommended solvent for sample dissolving is weak HILIC solvents such as ACN, MeOH, isopropanol, etc

Guidance for the mobile phases in HILIC mode:

- Always keep at least 5% polar solvent (such as 5% water phase buffer) in the mobile phase since this will ensure the silica gels packed in the HILIC mode cartridge (Claricep HILIC / NH2 / DIOL) to be always wetted by water.
- Mobile phase pH has a far greater impact on retention and selectivity in HILIC than in reversed phase separations. Ammonium Formate Buffer pH 3.2 or Ammonium Acetate buffer, pH 5.8 are most commonly used.
- Typical recommended range of buffer system concentration: 5-50mM in each phase
- Hexane, Ethyl Acetate, Dichloromethane, and mineral Ether cannot be used as storage solvents.
- Avoid major changes of pressure and temperature and any mechanical vibration during use.
- For 1500 - 5000g columns, O-ring can be used in alcohols, acetonitrile, ethyl acetate, acetone etc. It can swell slightly in petroleum ether or alkane solvent but will not cause leaking.
- It might appear that 1500 - 5000g columns have a little different surface color from other sizes, which can be caused by different batches of plastic material but won’t cause any decrease in performance.

For any additional questions visit:
Phenomenex.com/chat

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