This Data Sheet Contains Important Information About The Product.

Care and Use Guide for Discovery Zr-Carbon

Discovery Zr-Carbon comprises spherical, porous carbon-coated zirconia particles. It is ideal for the reversed-phase separation of positional isomers and diastereomers, and many other compounds. It complements the selectivity offering of the other zirconia-, silica, and polymer-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 14. It is a great alternative when C18 does not work.

Discovery Zr-Carbon product specifications:
Particle composition: carbon-coated zirconia
Particle size: 3 and 5 micron
Surface area: 30 m^2/g
Pore size: 300 Å
pH range: 1 - 14
Temperature range: < 100°C (Note: Special column hardware for operations between 100 and 200°C is available. Please inquire to our technical service.)
Pressure range: 4500 psi maximum

Care – Recommendations to maximize column lifetime

We recommend you reproduce the test chromatogram supplied with the column. If your results deviate significantly, please contact our technical services for some troubleshooting suggestions. Routinely retesting your column throughout its lifetime will also make sure you are developing the best possible methods.

Protection: Use a guard column packed with the same material as in the analytical column. This increases column life by preventing both mechanical and chemical fouling. Use HPLC grade solvents. Filter samples and mobile phases and be sure buffer precipitation does not occur upon mixing mobile phase components. Always use fresh mobile phase and prevent or be alert for microbial growth. Use an in-line filter (0.5 micron) in front of column to catch large particulates. Minimize pressure surges. Do not exceed 50% THF in the mobile phase.

Cleaning and regeneration:
Loss of efficiency or retention, or increased back pressure are indicators of column fouling. Carboxylic acids, fluoride, and phosphate all adsorb strongly to zirconia-based columns. They are easily removed by flushing the column with 20 column volumes of 0.1M ammonium hydroxide. If the column becomes fouled by adsorption of other compounds, you can attempt to remove the contaminants by flushing the column with 0.1M nitric acid at 50 – 90°C. The column can then be flushed with 0.1M ammonium hydroxide. When cleaned, the column should be flushed with 50:50 THF:CH_3CN at ambient temperature, then returned to normal operating conditions.

Column flushing and storage:
Discovery Zr-Carbon columns **should never be stored in phosphate buffers**. For overnight storage, flush the column with 50:50 organic modifier:water for 30 column volumes, followed by 100% CH_3CN for 10 column volumes, and finally 50:50 organic modifier:water again prior to storage overnight. For long-term storage, flush the column with 0.1M ammonium hydroxide first, followed by 50:50 organic modifier:water, 100% CH_3CN, and finally 50:50 organic modifier:water for 10 column volumes each. Re-equilibrate in 50:50 organic modifier:water when the column is put back in service.

Use – Recommended operating conditions

Organic modifiers: Discovery Zr-Carbon is compatible with any commonly-used organic modifier for HPLC (THF, CH_3CN, CH_3OH, isopropanol). However, THF up to 50% may give slightly better column efficiency. Do not exceed 50% THF. Because Discovery Zr-Carbon is slightly less hydrophobic than a silica-based C18, it requires typically 10-15% less organic modifier to obtain roughly the same retention as you would on a typical silica-based C_8 or C_{18}. **Caution:** Do not use PEEK tubing at temperatures above 100 °C, or with THF containing mobile phases.

Temperature: Column efficiency is significantly better at higher temperatures. If compound stability permits, we recommended to run between 50 - 75°C. The columns are stable up to 100°C. (Column hardware compatible with higher temperatures up to 200°C is available. Please inquire to our technical service.)

Flow rates: Discovery Zr-Carbon particles give low backpressure compared to their silica counterparts. We suggest you take advantage of this and the high run temperatures by using a flow rate of 3mL/min to significantly reduce the run time. **Caution:** Do not use PEEK tubing at temperatures above 100 °C, or with THF containing mobile phases.

Buffers: It is always good practice to use buffers in the mobile phase when analyzing ionizable compounds by HPLC. For basic (cationic) compounds, we recommend phosphate, acetate, citrate, carbonate/bicarbonate buffers on Discovery Zr-Carbon, not the amine buffers (like TEA) used on silica columns. A good choice is 10 – 25 mM ammonium phosphate, pH 7. However, this column is stable from 1 – 14 and you can use any buffer you like. For LC/MS work, we recommend 10 – 100 mM ammonium hydroxide/ammonium fluoride buffers or ammonium hydroxide/ammonium formate buffers at pH 10 – 12. For carboxylated or other acidic (anionic) compounds, we recommend adding 5 mM ammonium fluoride to the mobile phase. A commonly-used mobile phase for carboxylates is 10 – 25 mM ammonium phosphate, 5 mM ammonium fluoride, pH 6 – 8.

pH: Discovery Zr-Carbon columns are stable and give excellent results from pH 1 to 14. For basic compounds, experiment with high pH, and low pH for acidic compounds to maximize their hydrophobicity. At low pH (pH <4) do not add ammonium fluoride to the mobile phase as this can lead to the formation of HF.

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Method Development Guidelines for Discovery Zr-Carbon

General Method Development Guidelines
1. Start with >50% organic modifier in the mobile phase, and decrease in increments of 10% to achieve desired retention time.
2. Change organic modifiers (e.g. CH_3CN, CH_3OH, THF) to determine their effect on chromatographic selectivity, peak shape, and efficiency. Do not exceed 50% THF.
3. Ionic or ionizable compounds are influenced by buffering species, pH, and ionic strength. Study the effect of each on retention, selectivity, efficiency, and peak shape.
4. Increased operating temperature will decrease analysis time and usually improve peak shape and efficiency.

Neutral Compounds
1. Use mobile phases of CH_3CN or THF in water with at least 5% THF. Do not exceed 50% THF.
2. Adjust % organic modifier for optimum retention, efficiency, selectivity, and peak shape.
3. Use elevated operating temperatures when possible and as allowed by the stability of the analyte.
4. Use higher percentages of THF in the mobile phase for highly polar and polyaromatic compounds.

Acidic Compounds
1. Use at least 20mM phosphate buffer systems for the aqueous component of the mobile phase, using phosphoric acid as the phosphate source at very low pH.
2. The addition of a fluoride salt may be useful when operating above pH 4.
3. The use of ammonium salts of fluoride and phosphate is preferred over the sodium and potassium salts.
4. A minimum of 5% THF organic modifier is recommended for improved peak shape. Do not exceed 50% THF.
5. Discovery Zr-Carbon columns are stable even at pH 1.

Basic Compounds
1. Use at least 20mM phosphate buffer systems for the aqueous component of the mobile phase.
2. The use of the ammonium salt of phosphate is preferred over the sodium and potassium salts.
3. Discovery Zr-Carbon columns are stable even at pH 14.

Improving Difficult Separations
1. For very basic compounds with pKa's greater than 10, operate the column at the maximum temperature allowed by the HPLC system. When doing this, one may need to lower the organic content to maintain enough retention.
2. For zwitterionic compounds, increase the ionic strength of the buffer system and adjust the pH until a suitable separation is achieved, while operating at a high column temperature.

Recommendations for the Analysis of Ionizable and Non-ionizable Compounds on Discovery Zr-Carbon

➨ Best Performance 0 Acceptable Performance X Poor Performance

Non-ionizable compounds

	Organic Modifier			Temperature	
Compound	CH_3CN	THF	CH_3CN:THF mixtures	< 50°C	>50°C
N-Benzylformamide	0	0	0	0	➨
Benzylalcohol	0	0	0	0	➨
Phenol	0	➨	0	0	➨
3-Phenylpropanol	0	0	0	0	➨
p-Chlorophenol	X	➨	X	0	➨
Acetophenone	0	0	➨	0	➨
Benzonitrile	0	0	0	0	➨
Nitrobenzene	0	0	0	0	➨
Methylbenzoate	0	X	➨	0	➨
Anisole	➨	➨	0	0	➨

	Organic Modifier			Temperature	
Compound	CH_3CN	THF	CH_3CN:THF mixtures	< 50°C	>50°C
Benzene	0	➨	0	0	➨
p-Chlorotoluene	0	➨	0	0	➨
p-Nitrobenzyl chloride	0	➨	➨	0	➨
Benzophenone	X	0	0	0	➨
Bromobenzene	0	➨	0	0	➨
Napthalene	0	0	0	0	➨
p-Xylene	0	➨	➨	0	➨
p-Dichlorobenzene	0	➨	0	0	➨
Butylbenzene	0	➨	0	0	➨

Ionizable compounds

		Organic Modifier			Buffer pH			Temperature	
Compound Class	Example	CH_3CN	THF	CH_3CN:THF mixtures	< 4	4 to 9	> 9	< 50°C	>50°C
Anti-inflammatory drugs	Ibuprofen	0	0	0	➨			0	➨
Carboxylic acids	Chlorobenzoic acid	X	➨	0	➨			0	➨
Opioids	Codeine	X	0	0			➨	0	➨
Antidepressants	Diazepam	X	➨	➨		➨		0	➨
Steroids	Hydrocortisone	X	➨	0		0		0	➨
Anticonvulsants	Primidone	0	➨	➨		0		0	X
Phenols	Ethylparaben	X	➨	0				0	➨
Beta-blockers	Acebutolol	0	0	0			➨		➨
Antihistamines	Diphenhydramine	0	0	0			➨		➨

Note: Elevated operating temperatures improve peak shape and efficiency. Use temperatures as high as the compound's stability and operating system permit. Discovery Zr-Carbon columns are stable up to 100°C. (Column hardware compatible with higher temperatures up to 200°C is available. Please inquire to our technical service.)