

Care and Use Guide for Discovery Zr-PBD

Discovery Zr-PBD comprises spherical, porous zirconia particles with a durable coating of polybutadiene. It operates via a reversed-phase mechanism, but is less hydrophobic, so less organic solvent is required for elution. Discovery Zr-PBD 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 13.

Discovery Zr-PBD product specifications:

Particle composition: polybutadiene (PBD)-coated zirconia
Particle size: 3 and 5 micron
Surface area: 30 m²/g
Pore size: 300 Å
pH range: 1 - 13
Temperature range: < 100°C (Note: Special column hardware for operations between 100 and 150°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.

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 for 20 column volumes. When cleaned, the column should be flushed with 100% CH₃CN, CH₃OH, or isopropanol, then returned to normal operating conditions.

Column flushing and storage:

Discovery Zr-PBD columns **should never be stored in phosphate buffers**. For overnight storage, flush the column with 50:50 organic modifier:water for storage overnight. For long-term storage, flush the column with 0.1M ammonium hydroxide first, followed by 50:50 organic modifier:water. Re-equilibrate in 50:50 organic modifier:water when the column is put back in service.

Use – Recommended operating conditions

Organic modifiers: Discovery Zr-PBD is compatible with any commonly-used organic modifier for HPLC (THF, CH₃CN, CH₃OH, isopropanol). However, CH₃CN may give slightly better column efficiency. Do not exceed 50% THF. Because Discovery Zr-PBD 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 C₈ or C₁₈. **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. Extended temperatures up to 150°C is permitted with special hardware.

Flow rates: Discovery Zr-PBD 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-PBD, not the amine buffers (like TEA) used on silica columns. A good choice is 10 – 25 mM ammonium phosphate, pH 7. 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. Selectivity is modifiable through the addition of a strong Lewis base to the mobile phase such as fluoride, phosphate or hydroxide.

pH: Discovery Zr-PBD columns are stable from pH 1 to 13. For basic compounds, experiment with high pH, and low pH for acidic compounds to maximize their hydrophobicity. At low pH (pH <5) do not add ammonium fluoride to the mobile phase as this can lead to the formation of HF.

Method Development Guidelines for Discovery Zr-PBD

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. CH₃CN, CH₃OH, THF) to determine their effect on chromatographic selectivity, peak shape, and efficiency.
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. Any common RP-HPLC solvent may be used. However, CH₃CN usually provides the highest efficiency.
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.

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. (see below)
2. The addition of a fluoride salt may be useful when operating between pH 4 and pH 7.
3. The use of ammonium salts of fluoride and phosphate is preferred over the sodium and potassium salts.
4. Discovery Zr-PBD columns are stable even at pH 1.

Basic Compounds

1. Use buffer systems containing phosphate, acetate, fluoride, or hydroxide. (see below)
2. Use at least 20mM phosphate buffer systems for the aqueous component of the mobile phase.
3. The use of the ammonium salt of phosphate is preferred over the sodium and potassium salts.
4. Discovery Zr-PBD columns are stable even at pH 13.

Improving Difficult Separations

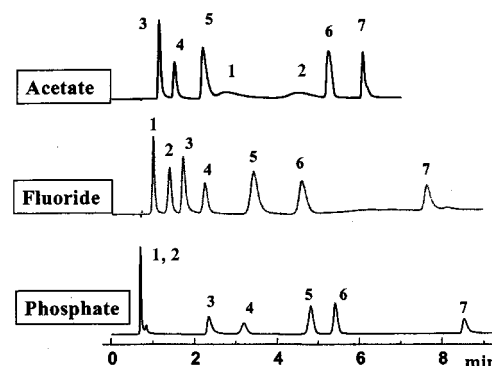
1. For very basic or very acidic compounds, the use of extreme pH conditions is encouraged to provide optimum separations without loss of column performance.

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.

Tips for optimizing the separation of basic compounds:

1. Retention is tunable by type of Lewis base (phosphate, fluoride, citrate, acetate, etc.) mobile phase additive.
2. Higher ionic strength lowers the retention of bases.
3. Higher pH increases the retention of bases. In phosphate buffers, higher pH decreases retention of bases.

Conditions: Discovery Zr-PBD 5µm, 5 cm x 4.6mm ID; Mobile phase: 30mM additive, 15mM TRIZMA, pH 7.5; Flow rate: 0.8mL/min; Temp: 40°C; Detection: UV 254nm; Sample: (1) chlorpropamide, (2) tolbutamide, (3) procainamide, (4) N-acetylprocainamide, (5) propionylprocainamide, (6) lidocaine, (7) quinidine



Tips for optimizing the separation of acidic compounds:

1. Use phosphate buffer.
2. Use acetonitrile or THF rather than methanol.
3. Use a small amount of fluoride (5mM).
4. Use low pH to increase retention by suppressing analyte ionization.
5. Higher temperature (>40°C) increases column efficiency.

Conditions: Discovery Zr-PBD 5µm, 5 cm x 4.6mm ID; Mobile phase: 25% CH₃CN, 75% 40mM additive, 5mM NH₄F; Flow rate: 0.6mL/min; Temp: 30°C; Detection: UV 254nm; Sample: (1) benzoic acid, (2) methoxybenzoic acid, (3) ethoxybenzoic acid, (4) propoxybenzoic acid

