

## RECOMMENDATIONS for USE, CLEANING and STORAGE of ZIRCONIA-BASED HPLC COLUMNS

### ZirChrom®-SAX

Thank you for purchasing this zirconia-based reversed phase high performance liquid chromatographic column from ZirChrom Separations. This product and/or its method of use is covered by one or more of the following patent(s): **US Patent No. 5,015,373, 5,108,597, 5,141,634, 5,205,929, 5,254,262, 7,897,798, Re: 34,910, 5,271,833, 5,346,619, 5,540,834, 6,846,410** and foreign equivalents. Additional patents are pending in the United States. We are sure you will be completely satisfied with its performance. In order to enjoy the tremendous benefits of its unique features compared to silica and polymer-based HPLC media, it is very important that you read the recommendations below. Please keep in mind that while this is anion-exchange column the substrate is zirconia, not silica, and the surface chemistry is completely different. If at any time you have a question about this product we invite you to visit our web site (<http://www.zirchrom.com>) where you will find a complete list of over 70 technical articles in peer reviewed journals on zirconia-based HPLC. In addition, our staff is always eager to help you with any aspect of using this column (1-866-STABLE-1).

#### Use:

1. Upon receipt, we suggest you duplicate the results on the enclosed chromatogram. You should be able to achieve a plate count of at least **90,000 plates/meter** (*specification for 150 x 4.6 mm i.d. format*) for nitrate under the operating conditions given on the chromatogram. Be sure to inject roughly the same amount of material as indicated in the chromatogram.
2. We very strongly advise that you use our columns at temperatures above ambient. We routinely use ZirChrom®-SAX columns at 50°C and find them to be stable at 80°C. We also recommend that you **increase the flow rate** at these super-ambient temperatures. **This will substantially increase the speed of analysis.** The backpressure of zirconia-based columns is remarkably low at room temperature and decreases substantially at 50°C and higher.
3. Operate the column in the pH range of 1 to 12.
4. Selectivity is modifiable through the addition of a strong Lewis base to the mobile phase (i.e. fluoride, phosphate, or hydroxide). Use ~10-20 mM of like chemical species, or fluoride or phosphate ion to improve peak shape (i.e. add acetic acid or phosphoric acid to mobile phase to improve peak shape of other organic acids).
5. To maximize the life of this ultra-durable column, we recommend the following precautions regarding day-to-day operation of the column.
  - ✓ **Always use a guard column.**
  - ✓ Clean up samples before injection (either filtering to remove particulates or solid phase extraction techniques).
  - ✓ Use HPLC grade solvents and filter all solutions before use.
  - ✓ Minimize pressure surges.
  - ✓ Use an in-line filter (0.5 micron) in front of column to catch large particulates.
  - ✓ Flush all buffers and salts from column before storage.

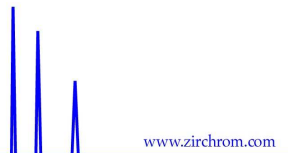
#### Cleaning/Regeneration:

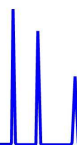
Carboxylic acids, fluoride and phosphate all adsorb strongly to zirconia-based columns. To fully remove these from the zirconia surface, or to remove any substance that may have fouled the column, use the following two-step cleaning protocol:

1. Flush the column with a high ionic strength mobile phase that contains a strong hard Lewis base (i.e. 0.1 molar ammonium phosphate dibasic + 0.5 molar ammonium sulfate at pH 8.0) for 50 column volumes at ambient temperature. Follow salt wash with 20 column volumes of pure water at ambient temperature.
2. Flush column with 100% water miscible organic solvent for 20 column volumes at ambient temperature. Follow organic wash with 20 column volumes of water at ambient temperature.

#### Storage:

Flush the column according to the cleaning/regeneration protocol before long-term storage. The ZirChrom®-SAX column should be stored in 90/10, 10 mM ammonium phosphate at pH 7.0/isopropanol.





A complete list of chromatography products offered by ZirChrom Separations:

### HPLC Columns

| Part # | Product Name     | Chromatographic Mode                 |
|--------|------------------|--------------------------------------|
| DB01   | Diamondbond®-C18 | C18 Modified Carbon Reversed-phase   |
| EZ01   | ZirChrom®-EZ     | Deactivated Reversed-phase           |
| MS01   | ZirChrom®-MS     | Deactivated Reversed-phase for LC/MS |
| TI01   | Sachtopore®-RP   | Reversed-phase (Titania)             |
| TI02   | Sachtopore®-NP   | Normal Phase (Titania)               |
| ZR01   | ZirChrom®-CARB   | Carbon Reversed-phase                |
| ZR02   | ZirChrom®-PHASE  | Normal Phase                         |
| ZR03   | ZirChrom®-PBD    | Reversed-phase                       |
| ZR04   | ZirChrom®-WCX    | Weak Cation-exchange                 |
| ZR05   | ZirChrom®-WAX    | Weak Anion-exchange                  |
| ZR06   | ZirChrom®-SAX    | Strong Anion-exchange                |
| ZR07   | ZirChrom®-SHAX   | Strong Hydrophilic                   |
| ZR08   | ZirChrom®-PEZ    | Cation-exchange                      |
| ZR09   | ZirChrom®-PS     | Reversed-phase                       |

### Specialty Products

| Part # | Product Name                    | Chromatographic Mode                 |
|--------|---------------------------------|--------------------------------------|
| AB01   | Rhinophase-AB                   | Pseudo-Affinity Phase for Anitbodies |
| BW01   | Advanced Buffer Wizard Software | 50 buffer systems (CD-ROM)           |
| MK01   | Ion-exchange Method Kit #1      | SAX, SHAX, WAX                       |
| MK02   | Ion-exchange Method Kit #2      | SAX, WCX, PEZ                        |
| MK03   | Reversed-phase Method Kit #1    | PBD, CARB, DB01                      |
| MK04   | Reversed-phase Method Kit #2    | EZ, CARB, PBD                        |
| NPZ    | Nonporous Zirconia              | 0.5, 1, 2, or 3 µm                   |
| ZRC01  | ZirChrom®-Chiral(S)LEU          | Pirkle Type chiral phase             |
| ZRC02  | ZirChrom®-Chiral(R)NESA         | Pirkle Type chiral phase             |
| ZRC03  | ZirChrom®-Chiral(S)NESA         | Pirkle Type chiral phase             |
| ZRC04  | ZirChrom®-Chiral(S)PG           | Pirkle Type chiral phase             |
| ZRC05  | ZirChrom®-Chiral(R)PG           | Pirkle Type chiral phase             |
| ZRC06  | ZirChrom®-CelluloZe             | Polysacchiride chiral phase          |

**Note: All chromatography products are available in microbore, analytical, semi-preparative and preparative column formats.**

