

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

### ZirChrom®-PHASE

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 a normal phase 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 **60,000 plates/meter** (*specification for 150 x 4.6 mm i.d. format*) for benzylcyanide under the operating conditions given on the chromatogram. Be sure to inject roughly the same amount of material as indicated in the chromatogram.
2. This column can be operated in a normal phase mode, an ion exchange mode, or a ligand exchange mode.
3. We very strongly advise that you use our columns at temperatures well above ambient. At a minimum, we urge you to set the column to **50°C**, but preferably to the highest temperature consistent with the stability of your analyte. We routinely use ZirChrom®-PHASE columns at 75°C and find them to be stable at 200°C. We also recommend that you set the **flow rate to 3 ml/min.** 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.
4. Operate the column at an acid/base concentration of 0.1 M or less. Avoid the use of concentrated hydrofluoric acid.
5. Selectivity of this phase is modifiable through the addition of a strong Lewis base to the mobile phase (i.e. fluoride, phosphate, or hydroxide). Prolonged (or high temperature) exposure to inorganic phosphates or fluoride will permanently modify the stationary phase. Column should be thoroughly flushed with 0.1 M NaOH followed by water to re-establish initial column performance.
6. 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.

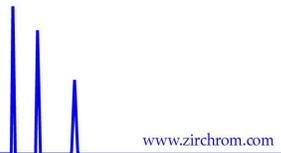
#### **Caution:**

Do not use PEEK tubing at temperatures above 100°C, or with THF containing mobile phases.

#### **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 three-step cleaning protocol:

**\*\*IMPORTANT\*\*** - During these steps you should remove your detector from the flow path to protect it from aggressive cleaning conditions.





1. Flush column with 0.1 M sodium hydroxide or tetramethyl ammonium hydroxide for 50 column volumes at ambient temperature. Follow base wash with 10 column volumes of water at ambient temperature.
2. Flush column with 0.1 M nitric acid for 50 column volumes at ambient temperature. Follow acid wash with 10 column volumes of water at ambient temperature.
3. Flush column with 100% organic solvent (i.e. ethanol) for 20 column volumes at ambient temperature.

**Storage:**

The ZirChrom®-PHASE column **should not be stored in a buffer**. We strongly suggest flushing the column with an organic solvent for 20 column volumes at ambient temperature prior to storage over night. Flush the column according to the cleaning/regeneration protocol before long-term storage.

**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.**

