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

DiamondBond®-C18

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, 8,137,548** 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 reversed-phase column the substrate is zirconia, not silica. 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 ethylbenzene 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 used with any common organic modifier (acetonitrile, methanol, tetrahydrofuran, isopropanol) but we find that **tetrahydrofuran gives somewhat better plate counts.** DiamondBond®-C18 is slightly less hydrophobic than common silica-based supports. For simple non-ionic compounds, we recommend that you use about **10-15% less organic modifier** to obtain roughly the same retention as you would on a typical C8 or C18 silica-based phase.
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 DiamondBond®-C18 columns at 75℃ and find them to be stable at 200℃. 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℃ and higher.
4. When chromatographing **basic compounds** on any stationary phase, a buffer must be used (**see ZirChrom Buffer Wizard at www.zirchrom.com**). We strongly advise the use of phosphate, and carboxylic acid (acetate, citrate, bicarbonate/carbonate) buffers, not the amine buffers used with silica columns. Our first choice for cationic drugs is **10-25 mM pH 7.0 ammonium phosphate buffers**. However, this column is stable from pH 1 to pH 14 and you can use any buffer you like in this pH range. **For LC-MS work**, we strongly advise use of 10-100 mM ammonium hydroxide/ammonium fluoride buffers or ammonium hydroxide/ammonium formate buffers at pH 10-12.
5. When carboxylated and other acidic molecules are chromatographed, we strongly advise the use of a buffer plus about 5 mM ammonium fluoride. Our favorite buffer for carboxylates is 10-25 mM ammonium phosphate plus 5 mM ammonium fluoride at pH 6-8. However, excellent results can be obtained at very low pH (pH < 1, without fluoride due to HF formation) and up to quite high pH (>11).

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.
- ✓ Always check the solubility of the buffer being used when mixing with organic mobile phases using an LC pump.

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 a mixture of 20/80 ACN/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 a mixture of 20/80 ACN/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 for 20 column volumes at ambient temperature. For ZirChrom®-PBD and ZirChrom®-PS, methanol, acetonitrile, isopropanol, and tetrahydrofuran are all adequate solvents. For ZirChrom®-CARB and DiamondBond®-C18, the same organic solvents can be used, however the solvent should contain at least 20% tetrahydrofuran.

Storage:
The DiamondBond®-C18 column ***should not be stored in phosphate buffer***. We strongly suggest flushing the column with 50/50 modifier-water for 30 column volumes, followed by flushing with 100% THF for 10 column volumes and re-equilibration with 50/50 modifier-water prior to storage over night. For long-term storage, we recommend flushing the column with 0.1M ammonium hydroxide first, followed by 50/50 modifier-water and 100% THF for 10 column volumes each, followed by re-equilibration with 50/50 organic modifier-water.

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.