General Care & Use Instructions for HPLC Columns packed by MZ-Analysentechnik GmbH

Please read this Care & Use information before using the column to ensure optimum column lifetime and reliability.

1. General Information

All HPLC columns from MZ Analysentechnik GmbH are individually manufactured and tested to meet strict specifications. Each HPLC column from MZ Analysentechnik GmbH comes with HPLC-Column Quality Certificate, which is specific to each individual column and contains many important information. Please keep this information for documentation purposes and future reference.

2. Sample Preparation

For optimum peak shape and sensitivity it is recommended to prepare the sample in the operating mobile phase or to use a mobile phase, that is a weaker solvent than the mobile phase. If possible, filter samples through a 0.2–0.45 µm syringe filter before injection.

3. Column Protection

To extend column lifetime and prevent contamination, the use of a guard column is recommended. Guard columns will protect the column from sample contaminants and highly retained solutes. For maximum column lifetime guard columns should be replaced at regular intervals, depending on the level of sample contamination.

4. HPLC Solvents/Mobile Phase

Use only HPLC grade solvents, filtered through a 0.2–0.45 µm membrane and degassed. Use only solvent additives (e.g. Ion-pairing reagents or buffer salts) of high purity (HPLC grade) or filter all aqueous buffers prior to use. The use of freshly prepared aqueous buffer solutions is recommended in order to minimise bacterial growth. Do not use mobile phases or compounds that chemically attack the bonded phase or silica. Never pump immiscible solvents sequentially through the column. The use of an online degassing unit is also recommended. For Amino–, Cyano–, Diol–, Ion-exchange– and the MZ-Aqua Perfect™ column the use of 100 % aqueous mobile phase is possible. Other stationary phases are not compatible with 100 % aqueous eluents and the water content should not exceed 95 %. For reversed phase separations typical eluents are acetonitrile, methanol or tetrahydrofurane in mixture with pure water or buffer. For normal phase separations typical eluents are n-hexane or n-heptane in mixture with an aliphatic alcohol (e.g. 2-propanol or ethanol).

5. Equilibration

Prior to measurement of samples the column must be rinsed with the eluent at the same flow rate and temperature as the method to be applied. Column equilibration is finished, when the baseline of the detector no longer shows a drift (generally after 10 column volumes).

6. pH-Range

The recommended operating pH range for columns from MZ-Analysentechnik GmbH is pH = 2.0 to 8.0. For PerfectSil ® Target HD columns, the usable range is extended from pH = 2.0 to 11.0. pH values outside this range can lead to irreversible damage of the columns. Please note that the usage of phosphate buffers at higher pH values (>7) may also cause damage to the column.

7. Pressure

Columns from MZ-Analysentechnik GmbH in general tolerate pressure of up to 400 bar (40 Mpa, 6,000 psi). In order to maximize column lifetime, operation at pressure higher than 300 bar should be avoided. Exposure of a column to rapid changes in back pressure may reduce column lifetime. Please note that for stationary phases with 300 Å or 1,000 Å pore width the maximum pressure should not exceed 300 bar or 200 bar, respectively.

8. Temperature

The maximum operating temperature is 60 °C. However, any temperature above ambient may have negative effect on column lifetime, which will vary depending on the pH and buffer conditions used. Variation of the temperature influences retention time and especially peak shape of analytes. Optimum temperature for a successful separation should be determined empirically.

9. Flow-Rate

The flow-rate is limited by the maximum column back pressure, which should not exceed the limit specified above. The flow-rate recommended for analytical columns is 0.2–2.0 mL/min. Typical flow rates for different column internal diameters (I.D.) are shown in the table on the right:

Column I.D.	Typical flow-rate
2.1 mm	0.2 mL/min
3.0 mm	0.4-0.5 mL/min
4.0 mm	0.7-0.8 mL/min
4.6 mm	1.0 mL/min

10. Storage

All HPLC columns should be stored in an appropriate solvent and capped tightly to prevent draining. Store column in a cool area, free from vibration using a special column storage cabinet or the shipping box. Flush columns after use with pH-neutral solvent to remove all possible contaminants and tag the column with the used storage solvent for later use (even for short-term storage). The original eluent shown on the initial column test chromatogram (see HPLC-Column Quality Certificate) is recommended as storage solvent. In general, the storage solvent should not contain more than 50% water to prevent bacterial and fungal growth. For Aminopropyl- and Diol-modified stationary phases we recommend 2-propanol, which is compatible with both Normal Phase and Reversed Phase Chromatography.

11. Regeneration and Cleaning Procedure

Contamination of the column by impurities from the sample or mobile phase can cause changes in peak shape, peak splitting, shifts in retention or high back-pressure. In some cases, these contaminants may be removed using a standard rinsing procedure employing different solvents, as shown in the following regenation schemes.

Regeneration scheme for reversed-phase columns (C30-, C18-, C8-, C4-, C1-, Cyano-, Amino-, Diol- and Phenyl-modified Phases)	Regeneration scheme for normal-phase columns (Silica-, Diol-, Nitro-, Cyano- and Amino-modified Phases)
20 column volumes of water/acetonitrile (95:5 v/v) 20 column volumes of acetonitrile 5 column volumes of 2-propanol 20 column volumes of *n*-heptane or *n*-hexane 5 column volumes of 2-propanol 20 column volumes of acetonitrile Re-equilibrate column with desired mobile phase	20 column volumes of *n*-heptane 5 column volumes of 2-propanol 20 column volumes of acetonitrile 20 column volumes of water 20 column volumes of acetonitrile 5 column volumes of 2-propanol 20 column volumes of *n*-heptane Re-equilibrate column with desired mobile phase

If an analytical HPLC column has dried out during storage, regeneration may be achieved via rinsing the column with about 10 column volumes of the storage solvent at a flow rate of 0.1–0.2 mL/min. Reflushing of the column in opposite flow direction is furthermore permitted, but should be carried out carefully at low flow rates