



# INSTRUCTION MANUAL FOR CD-SCREEN HPLC COLUMN 250 x 4 mm 5 µm analytical column

**Please read this manual before using CD-Screen column**

## COLUMN DESCRIPTION

**packing composition:** 4-nitrophenyl-urea bonded to 5 µm high purity silica

All column have a unique test, the shipping solvent is 100% Acetonitrile.

## RANGES OF OPERATING CONDITIONS:

### Temperature and pH of the eluent:

The safe temperature range depends on the pH of the eluent. Between pH 3.0-7.0 the highest temperature is 60 °C (140 °F). If the pH lower than 3.0 or higher than 7.0 the maximum temperature is 40 °C (104 °F).

Please note that formation of inclusion complex between the bonded nitrophenyl group and cyclodextrin derivatives becomes weaker at higher temperature, resulting in lower retention times and selectivity.

### Flow rate and pressure:

General recommendation: restrict back pressure to max. 250 bar (~3600 psi) adjusting flow rate to the appropriate value. In case of acetonitrile-water mixtures the maximum flow rate is about 1.5 ml/min, while using methanol-water the flow rate is limited in 1.0 ml/min, due to the higher viscosity of this eluent, especially in the 40-60% volume ratio interval.

Taking into account the slow kinetics of complex formation, the optimal flow rate is about 0.8 ml/min, the column shows the highest efficiency around this value.

### Solvent compatibility:

In reversed phase mode the eluent composition is not restricted to methanol-acetonitrile-water mixtures, the column is compatible with all of water-miscible solvents, e.g. ethanol, 2-propanol, dioxane, tetrahydrofurane, pyridine, acetone.

If the cyclodextrin derivative is soluble in organic solvents it is advisable to use CD-Screen column in polar organic or normal phase mode in order to achieve better selectivity.

Hexane, ethers, acetone, chloroform, dichloromethane, dioxane, tetrahydrofurane can be applied as eluent.

General rule: Switching between normal phase and reverse phase mode it is necessary to flush the column with an intermediate solvent, e.g. ethanol, 2-propanol or tetrahydrofurane. Please note that in case of 2-propanol the flow rate must be adjusted in accordance with pressure limitation, due to the high viscosity of this solvent. In general, 50 ml of intermediate solvent is enough to remove the previously used eluent from the column.

## Buffer solutions

pH 2.5-4.5: 50 mM Potassium-dihydrogen-phosphate – Phosphoric acid

pH 4.5-7.5: 50 mM Potassium-dihydrogen-phosphate – Sodium-hydroxyde

pH 2.5-7.5: 50 mM Phosphoric acid – Triethylamine

In case of ELS or MS detection Acetic acid, Formic acid, Trifluoro acetic acid and volatile Triethylamine salts of these can be used.

An abrupt change to high organic solvent content could result in buffer salt precipitation.

The risk is particularly high in case of gradient elution, because precipitation can occur in the column, deteriorating packing bed integrity. In order to avoid this problem try to use 80-90 v/v% organic solvent as the eluent „B” and triethylamine-phosphate buffers instead of alkali phosphates.

## Storage

Before disconnecting the column from the HPLC, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers.

If the column has become contaminated with non-eluted components, wash it with 100% acetonitrile for two hours at 0.3 ml/min. Alternatively, if the non-eluting components are more soluble in methanol, this solvent may be used for the washing step.

All salts must be flushed out from the HPLC system and column before changing to 100% acetonitrile or 100% methanol.

We suggest to use 100% acetonitrile to store the column.

## IMPORTANT!

Our HPLC columns are designed according to the Waters standard. SS ferrules and tubes previously fitted to another column can cause dead volume due to the different fixed position of the ferrule on the capillary tube. In order to avoid above situation, use PEEK ferrules, which move freely on the capillary tube and can fit perfectly tube and can fit perfectly to the column end fitting.

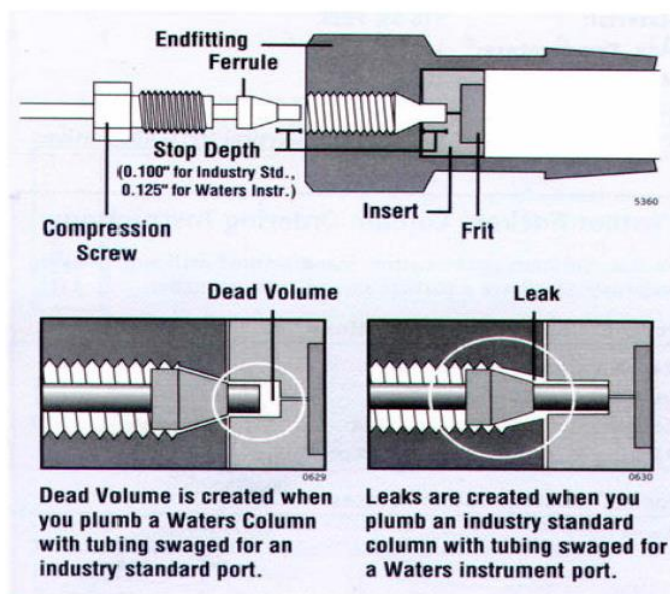


Figure 2 - Problems that Arise from Mismatched Stop Depth