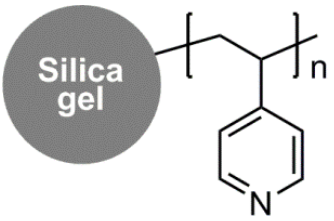
INSTRUCTION MANUAL FOR DCpak® P4VP

Please read these instructions completely before using this column

Column Description

DCpak® P4VP
Poly(4-vinylpyridine) **immobilized** on **3µm, 5µm, and 10µm silica** 

Shipping solvent: **100% Ethanol**

Every column has been examined and quality control tested before shipping. Please refer to the Column Performance Report and test parameters for results.

CAUTION

The column is designed for 34.35MPa maximum pressure and for 30MPa daily pressure.
Please use the column **neither** at a pressure over 30MPa **nor** at a temperature over 40°C.

Please flush the residual solvent in the SFC instrument with a recommended mobile phase (see page 2) before connecting the column to the instrument. Please be sure to flush the auto-sampler, syringe, needle, and injection loop as well.

Operating Conditions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical columns	**50 x 3.0 mm i.d.** **100 x 3.0 mm i.d.** **150 x 3.0 mm i.d.** Analytical columns	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical columns	**250 x 10 mm i.d.** **250 x 20 mm i.d.** **250 x 30 mm i.d.** **250 x 50 mm i.d.** Semi-prep/prep columns
Column Fittings	Waters			
Flow Rate Direction	As indicated on the column label			
Pressure Limitations①	30MPa (~ 305 kgf/cm^2 or ~ 4350 psi)			
Temperature②	0 to 70°C			
pH Range②	2.0 to 8.0			

①Pressure means the pressure at the column head, which is nearly equal to the pump pressure. The recommended back pressure regulator (BPR) setting is 8 – 20MPa. If the BPR setting is too low, an unstable chromatogram may result.
② When this column is used at pH > 7, the temperature should be maintained between 5ºC and 25ºC, and the use of guard cartridge is essential to maximize column life.

Important Notice

- **This column is not for chiral separations.**
- **Do not attempt to disassemble the column.**
- **P4VP can be used with all ranges of organic miscible solvents. To switch from reversed-phase or HILIC to normal phase or SFC and vice versa, the columns should be carefully flushed with miscible solvent, such as ethanol and 2-propanol.**
- **This instruction sheet for DCpak® P4VP is not applicable to any other Daicel column.**

Please contact your local Chiral Technologies office for further assistance before trying any solvents not mentioned below.

Method Development / SFC Mode

A – Mobile Phase

	CO_2/co-solvent
Composition	**100/0 to 0/100**

- Methanol is typically used as a co-solvent. Ethanol, 2-propanol, ethyl acetate, THF, and dichloromethane can also be used.
- The eluotropic strength of the alcoholic co-solvents are methanol>ethanol>2-propanol if the same volume percentage is applied. This tendency becomes remarkable for a polar analyte.
- A higher co-solvent content results in a shorter retention time.
- A mixed co-solvent of the above organic solvents can also be applied. When an **aprotic** co-solvent is employed, the addition of alcohol in a small amount may improve peakshape.
- An increase of the co-solvent content increases the column head pressure. **Pressure should not exceed 30 MPa**.

B – Method Development - Screening

When developing methods, we would recommend a screening approach. The typical screening starting condition is described below.

Flow Rate ①	2.0 to 3.0 mL/min (when column i.d. is 4.6 mm)
Co-solvent	Methanol
Gradient Condition (% of Co-solvent)	2 to 90% in 10 min
Back Pressure	10 to 15 MPa

① Please see conversion table below for other i.d. columns.

- Generally, a gentle gradient slope improves resolution, and a steep gradient slope accelerates elution.
- Based on the gradient screening results, further optimization can be performed by considering the ratio of mobile phase, gradient slope, column temperature, etc.
- When polar analytes are not eluted, the addition of water (up to 5%) can be helpful.

C – Additives

- Initial method development can be performed without the addition of any additives. If needed, the recommended additives as illustrated in the table may help sharpen the peak shape.
- Typical concentration is 0.1 vol% of the total mobile phase. (e.g. use co-solvent containing 0.5% of additive if CO_2/ co-solvent ratio is 80/20 v/v).

Additive for basic analyte	Additive for acidic analyte
Ammonium formate, Ammonium acetate ~0.1vol% of total mobile phase	
Diethylamine (DEA)	Trifluoroacetic acid (TFA)

- After a basic or acidic additive has been used, wash the column with more than 10 column volumes of mobile phase without additive, and then flush the column with ethanol.

Method Development / Normal Phase Mode

A - Method Development -Screening

The typical screening condition is described below.

Polar Organic Solvent	2-propanol, Ethanol, Methanol ①, Acetonitrile, etc.
Nonpolar Organic Solvent	n-Hexane ①, n-Heptane ①, Methyl *tert*-butyl ether, etc.
Typical Gradient Screening ②	Polar Organic Solvent / Non-polar Organic Solvent 5/95 to 95/5 in 20 min.
Flow Rate ③	1.0 mL/min (when column i.d. is 4.6 mm)

① MeOH and alkane (e.g. n-hexane, n-heptane, and so on) are NOT miscible, especially at low temperature. Please confirm miscibility before introduction to the column.
② Lowering the column temperature may increase the retention time and the selectivity. Increasing the column temperature and decreasing the flow rate may increase the resolution.
③ Please see conversion table below for other i.d. columns.

B - Additive

- Initial method development can be performed without the addition of any additives. If needed, the recommended additives as illustrated in the table may help sharpen the peak shape.
- Typical concentration is 0.1% of the total mobile phase.

Additive for basic analyte	Additive for acidic analyte
Ammonium formate, Ammonium acetate ~0.1vol% of total mobile phase	
Diethylamine (DEA)	Trifluoroacetic acid (TFA)

Method Development / Reversed Phase Mode

CAUTION! The use of strongly basic conditions (pH >8) must be avoided, as they are known to damage the silica gel matrix.

A - Method Development -Screening

The typical screening condition is described below.

	Acidic Analytes	Neutral Analytes	Basic Analytes ④
Aqueous Solution	Formic acid aq.(pH 2.0)	Water	20 mM Ammonium formate aq. (pH 8.0, adjusted with a basic additive)
Organic Modifier ①	Acetonitrile or Methanol or Ethanol or 2-Propanol or Tetrahydrofuran		
Typical Gradient Screening ②	Aqueous Solution / Organic Solution 95/5 to 5/95 in 20 min.		
Flow Rate ③	0.5 to 1.0 mL/min (when column i.d. is 4.6 mm)		

① It is recommended to use acetonitrile for initial screening. The use of alcohols results in significantly high pump pressure compared to acetonitrile due to their high viscosity.
② Lowering the column temperature may increase the retention time and the selectivity. Increasing the column temperature and decreasing the flow rate may increase the resolution. High percentages of organic modifier in the mobile phase may precipitate the buffering salt from the solution, and lead to subsequent clogging of the column.
③ Please see conversion table below for other i.d. columns.
④ To maximize column life the use of a guard cartridge is essential when basic conditions are employed.

CAUTION! The use of strong basic conditions (pH > 8) must be avoided, as they are known to damage the silica gel matrix. When these columns are used at pH > 7, the temperature should be maintained between 5ºC and 25ºC for maximum column life.

Method Development / HILIC Mode

A - Method Development - Screening

The typical screening condition is described below.

	Acidic Analytes	Basic Analytes ④
Additives	Ammonium formate or Ammonium acetate (typically 20 mM)	
	Formic acid Acetic acid	Ammonium hydroxide
Organic Modifier ①	Acetonitrile or Tetrahydrofuran or Acetone	
Typical Gradient Screening ②	Aqueous Solution / Organic Modifier 5/95 to 40/60 in 20 min.	
Flow Rate ③	0.5 to 1.0 mL/min (when column i.d. is 4.6 mm)	

① It is recommended to use acetonitrile for initial screening.
② Lowering the column temperature may increase the retention time and the selectivity. Increasing the column temperature and decreasing the flow rate may increase the resolution. High percentages of organic modifier in the mobile phase may precipitate the buffering salt from the solution, and lead to subsequent clogging of the column
③ Please see conversion table below for other i.d. columns.
④ To maximize column life, the use of a guard cartridge is essential when basic conditions are employed.

Sample Preparation

CAUTION! The use of strong basic conditions (pH > 8) must be avoided, as they are known to damage the silica gel matrix. When these columns are used at pH > 7, the temperature should be maintained between 5ºC and 25ºC for maximum column life.

The sample should be dissolved in the mobile phase co-solvent, i.e. methanol, ethanol, etc., and should be filtered through a membrane filter of approximately 0.5µm porosity.

Gradient and LC

Analysis can be performed by gradient elution, however baseline instability may occasionally occur, in particular after using basic or acidic additives. Before performing a gradient analysis, complete a "test run" to verify baseline stability.

Column Care / Maintenance

- After performing analyses which contain additives, it is good practice to flush the column with mobile phase which does not contain any additives. If removing the column from the system, flush with 100% ethanol or isopropanol first, and then remove the column following the notes below.
- Remove the column from the instrument **ONLY** after the inner pressure is completely released. Removing the column under a high inner pressure may cause hazards by rapid releasing CO_2, and can cause a deterioration of the column seal. Be sure to slowly loosen the connection to avoid possible release of CO_2.
- The column can be stored long term at ambient temperature.
- If reproducibility has been compromised, clean the column with more than 10 column volumes of ethanol at 1.0 mL/min. In addition, if the reproducibility has been compromised **AND** trifluoroacetic acid has been used repeatedly as an additive, it might be necessary to perform the column cleaning with 0.1vol% diethylamine in the mobile phase.

Conversion Table

Column ID vs Flow Rate

Column ID (mm)	2.1	3.0	4.6	10	20	30
Flow Rate (mL/min)	0.21	0.43	1.0	4.7	19	43

Pressure

MPa	**bar**	**kg/cm²**	**psi**
1	10	10.197	145.038
0.1	1	1.020	14.504
9.807×10-2	0.981	1	14.223
6.895×10-3	6.895×10-2	7.031×10-2	1

Operating this column in accordance with the guidelines outlined here will result in a long column life.

⇨ If you have any questions about the use of this column, or encounter a problem, contact:

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