

## **REGENERATION PROCEDURES FOR CHIRALPAK® IA, IB, IB N, IC, ID, IE, IF, IG, IH, IJ, and IK**

**The following regeneration procedures are ONLY intended for CHIRALPAK® immobilized-type columns. These procedures should never be used for any CHIRALCEL® or CHIRALPAK® coated-type columns.**

### **Regeneration Overview**

Following the extensive use of a column in multiple solvents, there may be a change in separation reproducibility. In order to ensure consistent performance, a regeneration method may be implemented to eliminate any change in chiral recognition due to the history of the column (mobile phases, additives...).

Because of the unique characteristics of each phase, different regeneration procedures have been developed to achieve optimal results. The following procedures can be used on all inner diameter columns and column lengths. Please see the specific section for the particle size column you are attempting to regenerate for important adjustments related to flow rate and time.

These procedures are also highly recommended when switching from reversed-phase (RP) to normal phase (NP) or SFC.

**If there are any questions or concerns about performing these procedures, please contact [Technical Support](#) before proceeding.**

### **Sub-2 µm Regeneration**

- 1.) For CHIRALPAK® IA-U and ID-U
  - Flush the column with ethanol at 0.21 ml/min for 30min, followed by N,N-dimethylformamide (DMF) at 0.17 ml/min for 240min.
  - Flush the column with ethanol at **0.02mL/min(\*)** for 600min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.21 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 2.) For CHIRALPAK® IB-U and IC-U
  - Flush with ethyl acetate (EtOAc) at 0.42 ml/min for 30 min (if a mobile phase containing additives was used, flush for 2 hours or more), plug both ends of the column, and let stand at room temperature for 48 hours or more.
  - Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.21 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 3.) For CHIRALPAK® IG-U
  - Flush with ethyl acetate (EtOAc) at 0.42 ml/min for 60 min (if a mobile phase containing additives was used, flush for 2 hours or more) at room temperature.
  - Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase

Instruction Manual) at 0.21 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

- 4.) For CHIRALPAK® IH-U
  - Flush with ethanol at 0.21 ml/min for 30 min, followed by N,N-dimethylformamide (DMF) at 0.13 ml/min for 3 hours.
  - Flush with ethanol at **0.13 ml/min(\*)** for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.21 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

*\* This low flow rate is critical for column performance*

## 3 µm Regeneration

### **Analytical Columns (2.1, 3.0, and 4.6 mm i.d.)**

The following procedures are for 4.6 mm inner diameter analytical columns, but can be used for all analytical column dimensions by scaling the flow rate proportional to the cross-sectional area (i.e a 4.6 mm i.d. column flowing at 1 ml/min has an equivalent flow rate of 0.43 ml/min on 3.0 mm i.d. column, and 0.21 ml/min on a 2.1 mm i.d. column):

- 1.) For CHIRALPAK® IA-3, ID-3, and IF-3
  - Flush the column with ethanol at 0.5 ml/min for 30min, followed by N,N-dimethylformamide (DMF) at 0.4 ml/min for 240 min.
  - Flush the column with ethanol at **0.05 ml/min(\*)** for 600 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 2.) For CHIRALPAK® IB-3, IB N-3, and IC-3
  - Flush with ethyl acetate (EtOAc) at 1 ml/min for 30 min (if a mobile phase containing additives was used, flush for 2 hours or more), plug both ends of the column, and let stand at room temperature for 48 hours or more.
  - Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 1 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 3.) For CHIRALPAK® IE-3 and IH-3
  - a. Flush the column with ethanol at 0.5 ml/min for 30min, followed by N,N-dimethylformamide (DMF) at 0.3 ml/min for 180 min.
  - b. Flush the column with ethanol at 0.3 ml/min for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 4.) For CHIRALPAK® IG-3
  - Flush with ethyl acetate (EtOAc) at 1 ml/min for 60 min (if a mobile phase containing additives was used, flush for 2 hours or more) at room temperature.
  - Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 1 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 5.) For CHIRALPAK® IJ-3
  - Flush with ethanol at 0.5 ml/min for 30 min, followed by dichloromethane (DCM) at 0.3 ml/min for 3 hours.
  - Flush with ethanol at **0.05 ml/min(\*)** for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

*\* This low flow rate is critical for column performance*

## 5 $\mu$ m Regeneration

### **Analytical Columns (2.1, 3.0, and 4.6 mm i.d.)**

The following procedures are for 4.6 mm inner diameter analytical columns, but can be used for all analytical column dimensions by scaling the flow rate proportional to the cross-sectional area (i.e a 4.6 mm i.d. column flowing at 1 ml/min has an equivalent flow rate of 0.43 ml/min on 3.0 mm i.d. column, and 0.21 ml/min on a 2.1 mm i.d. column):

- 1.) For CHIRALPAK® IA, ID, IE, IF, and IH
  - Flush the column with ethanol at 0.5 ml/min for 30min, followed by N,N-dimethylformamide (DMF) at 0.3ml/min for 180min.
  - Flush the column with ethanol at **0.3mL/min(\*)** for 50min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 2.) For CHIRALPAK® IB, IB N-5, IC, and IK
  - Flush with ethyl acetate (EtOAc) at 1 ml/min for 30 min (if a mobile phase containing additives was used, flush for 2 hours or more), plug both ends of the column, and let stand at room temperature for 48 hours or more.
  - Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 1 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 6.) For CHIRALPAK® IG
  - Flush with ethyl acetate (EtOAc) at 1 ml/min for 60 min (if a mobile phase containing additives was used, flush for 2 hours or more) at room temperature.
  - Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 1 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 7.) For CHIRALPAK® IJ
  - Flush with ethanol at 0.5 ml/min for 30 min, followed by dichloromethane (DCM) at 0.3 ml/min for 3 hours.
  - Flush with ethanol at **0.3 ml/min(\*)** for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

*\* This low flow rate is critical for column performance*

### **Semi-Prep and Preparative Columns (1, 2, 3 and 5 cm i.d.)**

For Semi-Prep and Preparative columns, a similar procedure as above can be used, but the time of each flush needs to be adjusted. The following procedures are for 1 cm semi-prep columns, but can be used for all semi-prep column dimensions by scaling the flow rate proportional to the cross-sectional area (i.e a 1 cm i.d. column flowing at 1 ml/min has an equivalent flow rate of 4.5 ml/min on 2 cm i.d. column, 9 ml/min on a 3 cm i.d. column, and 25 ml/min on a 5 cm i.d. column):

- 1.) For CHIRALPAK® IA, ID, IE, IF, and IH
  - a. Flush with ethanol at 2 ml/min for 30 min, followed by N,N-dimethylformamide (DMF) at 1.2 ml/min for 3 hours.
  - b. Flush with ethanol at **1.2 ml/min(\*)** for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 2 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.
- 2.) For CHIRALPAK® IB, IB N-5, IC, and IK
  - a. Flush with ethyl acetate (EtOAc) at 4 ml/min for 30 min (after flushing with mobile phase containing

additives, flush for 2 hours or more), plug both ends of the column, and let stand at room temperature for 48 hours or more.

- b. Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 2 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

3.) For CHIRALPAK® IG

- a. Flush with ethyl acetate (EtOAc) at 4 ml/min for 60 min (after flushing with mobile phase containing additives, flush for 2 hours or more) at room temperature.
- b. Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 2 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

4.) For CHIRALPAK® IJ

- Flush with ethanol at 2 ml/min for 30 min, followed by dichloromethane (DCM) at 1.2 ml/min for 3 hours.
- Flush with ethanol at **1.2 ml/min(\*)** for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 2 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

*\* This low flow rate is critical for column performance*

**\* For regeneration procedures of 10 or 20 µm immobilized columns, please contact us at the corresponding email address or telephone number below. \***

⇒ If you have any questions about the use of these columns, or encounter a problem, contact:

In the USA: [questions@cti.daicel.com](mailto:questions@cti.daicel.com) or call 800-6-CHIRAL

In the EU: [cte@cte.daicel.com](mailto:cte@cte.daicel.com) or call +33-388-79-5200

In India: [chiral@chiral.daicel.com](mailto:chiral@chiral.daicel.com) or call +91-84 1866 0700 & 703

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