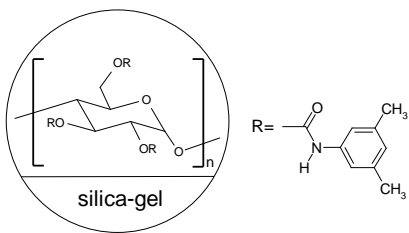
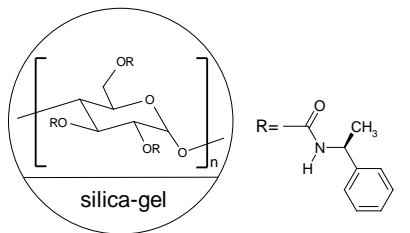
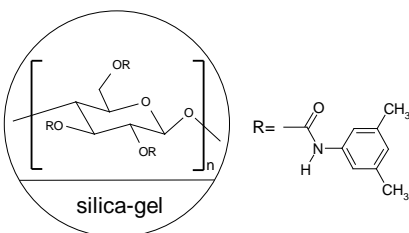
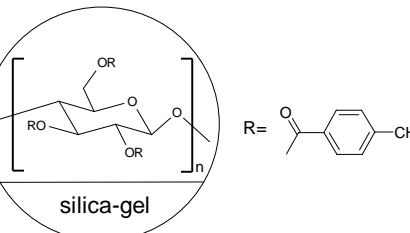


INSTRUCTION MANUAL FOR CHIRALPAK[®] AD-3, AS-3 and CHIRALCEL[®] OD-3, OJ-3

Please read this instruction sheet completely before using these columns

Column Description

<p style="text-align: center;">CHIRALPAK[®] AD-3</p> <p style="text-align: center;">Amylose tris(3,5-dimethylphenylcarbamate) coated on 3 μm silica-gel</p> <div style="text-align: center;">  <p style="margin-left: 100px;">silica-gel</p> </div>	<p style="text-align: center;">CHIRALPAK[®] AS-3</p> <p style="text-align: center;">Amylose tris((S)-α-methylbenzylcarbamate) coated on 3 μm silica-gel</p> <div style="text-align: center;">  <p style="margin-left: 100px;">silica-gel</p> </div>
<p style="text-align: center;">CHIRALCEL[®] OD-3</p> <p style="text-align: center;">Cellulose tris(3,5-dimethylphenylcarbamate) coated on 3 μm silica-gel</p> <div style="text-align: center;">  <p style="margin-left: 100px;">silica-gel</p> </div>	<p style="text-align: center;">CHIRALCEL[®] OJ-3</p> <p style="text-align: center;">Cellulose tris(4-methylbenzoate) coated on 3 μm silica-gel</p> <div style="text-align: center;">  <p style="margin-left: 100px;">silica-gel</p> </div>

Shipping solvent: n-Hexane / 2-Propanol solvent mixture (90:10 v/v)

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.

CAUTION

The entire HPLC system including the injector and the injection loop must be flushed with a solvent compatible with the column and its storage solvent prior to connecting. Many of the solvents commonly used in HPLC eluents such as acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride and THF may DESTROY the chiral stationary phase if they are present, even in residual quantities, in the system.

If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed and the relevant solvent lines flushed.

Operating Conditions

	50 x 4.6 mm i.d. Analytical columns	100 x 4.6 mm i.d. Analytical columns	150 x 2.1 mm i.d. Analytical columns	150 x 4.6 mm i.d. Analytical columns	250 x 4.6 mm i.d. ① Analytical columns
Flow rate direction	As indicated on the column label				
Typical flow rate ②	0.5 to 5 ml/min	0.5 to 5 ml/min	0.1 to 0.5 ml/min	0.5 to 5 ml/min	0.5 to 3 ml/min
Temperature	0 to 40°C				

☞ **NOTES:** The column packing is stable to HPLC pressures.
At a given temperature, the column back pressure is linearly proportional to the flow rate.

- ① The use of the longer column may be an efficient resort for difficult resolution of enantiomers.
- ② Flow rates in the range of 0.5-1.0ml/min are recommended for difficult resolution of enantiomers (search of the best resolution).
Flow rates superior to 1.0ml/min, preferably 3.0-5.0 ml/min, are advised for fast analyses.

Operating Procedure

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

A - Mobile Phases

	Alkane ^① / 2-Propanol ^②	Alkane ^① / Ethanol ^②	Alkane ^① / MeOH ^③	MeOH ^④ + ^⑤	CH ₃ CN ^⑤ + ^⑥ <u>No alkane at all</u>
CHIRALPAK® AD-3 CHIRALPAK® AS-3 CHIRALCEL® OD-3 CHIRALCEL® OJ-3	100/0 to 0/100	100/0 to 0/100	100/0 to 85/15	0 to 100% EtOH or CH ₃ CN in MeOH	0 to 100% EtOH or MeOH in CH ₃ CN

- ① Alkane: n-Hexane or iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
- ② - The retention is generally shorter with Ethanol than with 2-Propanol.
- The retention is generally shorter with higher alcohol contents.
- The use of other alcohols such as 1-Propanol, 1-BuOH, 2-BuOH etc...is possible, but effectiveness cannot be predictable.
- ③ Due to limited miscibility of MeOH in Alkane, it is necessary to add an appropriate volume of EtOH together with MeOH in order to obtain homogenous solvent mixtures.
A maximum of 5% MeOH in n-Hexane only may be used without adding EtOH.
- ④ Ideal starting conditions: MeOH/EtOH 50:50 (v/v) when alcohol mixtures are required.
- ⑤ The use of polar solvents such as 100% Methanol or 100% Acetonitrile is possible with CHIRALPAK® AD-3/AS-3 and CHIRALCEL® OD-3/OJ-3 columns. Nevertheless once the column is transferred to a polar mode **it should be dedicated to this specific application.**

To safely transfer the column from Hexane to Methanol or Acetonitrile **it is strongly recommended to use 100% EtOH as a transition mobile phase** at 0.5 ml/min.

- ⑥ The column needs to be thoroughly washed with Acetonitrile (~ 10 column volumes) prior to the first use in this solvent as mobile phase.

B – Additives

For basic samples or acidic samples, it is necessary to add an additive into the mobile phase in order to achieve the chiral separation.

- ⑦ For primary amines mainly
- ⑧ For primary amino alcohols mainly

Basic Samples require Basic additives	Acidic Samples require Acidic additives
DEA Butyl amine ^⑦ Ethanol amine ^⑧	TFA CH ₃ COOH
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

Column Care / Maintenance

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
 - ❑ Samples should be dissolved in the mobile phase and should be filtered through a membrane filter of approximately 0.5µm porosity.
 - ❑ For alkane containing mobile phases, flush the column with Storage Solvent (Hexane / 2-Propanol 9:1) when stored for more than one week.
 - ❑ For columns dedicated to polar solvents, flush the column with the regular mobile phase without the additive.
- ☞ When washing is required, use pure Ethanol at 0.5 ml/min for 1 to 3 hours. The column can be heated at 40°C for a more efficient cleaning.

Important Notice

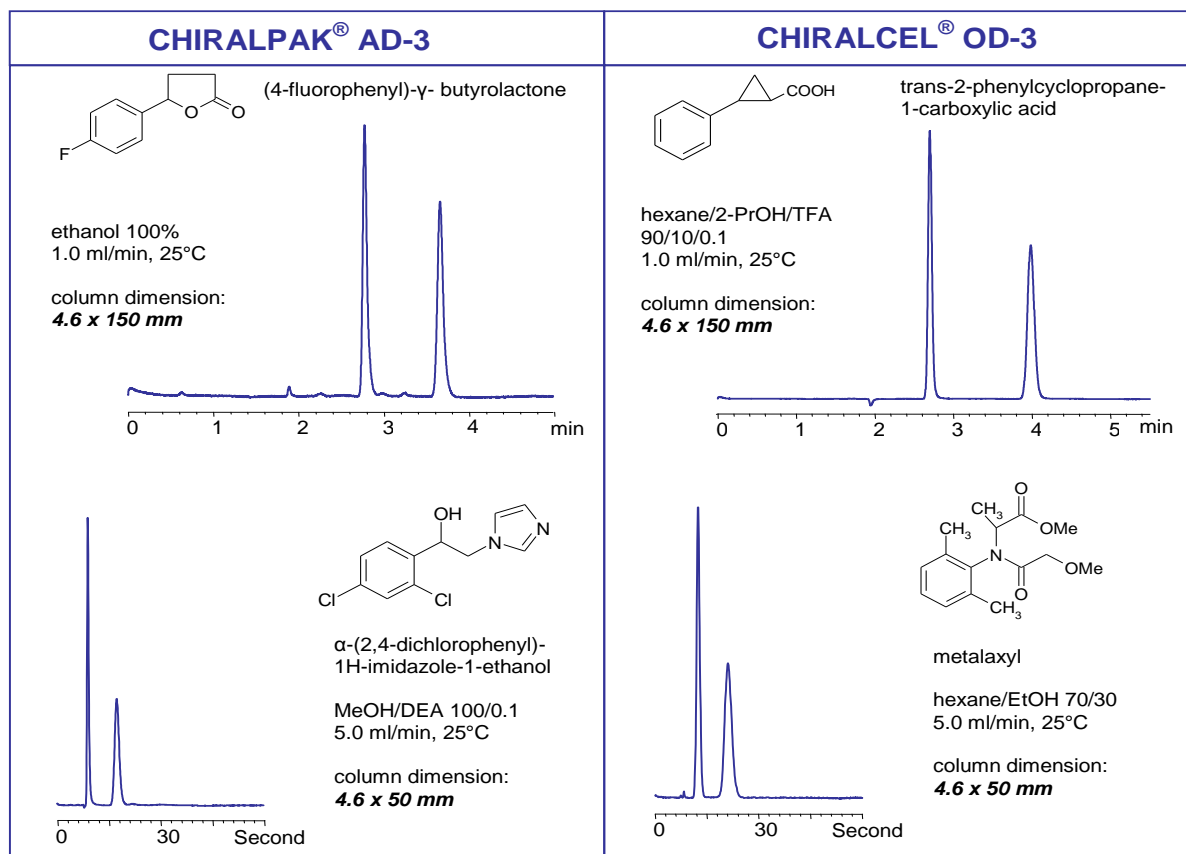
⇒ STRONGLY BASIC solvent additives or sample solutions MUST BE AVOIDED, because they are likely to damage the silica gel used in these columns.

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

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

In the USA: questions@chiraltech.com or call 800-6-CHIRAL
In the EU: cte@chiral.fr or call +33 (0)3 88 79 52 00
In India: chiral@chiral.daicel.com or call +91-40-2338-3700

Examples of HPLC applications



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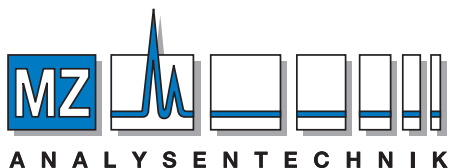
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