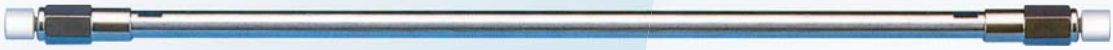
CHIRALPAK®IA
Revolution

Pure
Simplicity

NEW NEW NEW NEW



CHIRALPAK® IB

A new tool in a revolutionary generation of chiral stationary phases

- Complementary chiral recognition to CHIRALPAK® IA
- Immobilized version of CHIRALCEL® OD
- Extended mobile phase versatility
- Enhanced sample solubility
- Robustness

DAICEL CHEMICAL INDUSTRIES, LTD.

CHIRALPAK®IB is a new tool in a revolutionary generation of polysaccharide-derived chiral stationary phases from DAICEL Chemical Industries Ltd. This new packing material, designed for analytical and semi-preparative separations of enantiomers, is the immobilized version of CHIRALCEL® OD and plays a complimentary role to CHIRALPAK®IA in the separation of enantiomers with all kinds of miscible solvent systems

The immobilization of polysaccharide derivatives on a matrix has been considered as an evolutionary approach to implement universal solvent compatibility on these highly selective chiral stationary phases (CSPs) for enantioseparation. This broadens the range of solvents to be used as mobile phases, thereby introducing new selectivity profiles and beneficial CSP characteristics. In this context, Daicel Chemical Industries Ltd. is extending its produce line from the originally coated CSPs to the immobilized ones using proprietary immobilization technologies. CHIRALPAK© IA, a 3,5-dimethylphenyl carbamate derivative of amylose immobilized on the silica, was the first of this innovative generation of CSPs. CHIRALPAK© IB, the second member of this series, is now commercially available.

The chiral selector in CHIRALPAK© IB is of the same nature as in CHIRALCEL© OD i.e. tris (3,5-dimethylphenylcarbamate) derivatized cellulose. CHIRALCEL© OD is made by physical coating of the polymer on a silica gel, whereas the chiral selector in CHIRALPAK© IB is immobilized on the support.

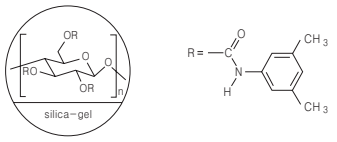


Figure 1. Packing composition of CHIRALPAK© IB

Because of their coated nature, the traditional polysaccharide-derived CSPs can only be used with a limited range of solvents as mobile phases or mobile phase components. In the case of CHIRALCEL© OD, for instance, we recommend the so-called standard mobile phases such as alkane/alcohols, pure alcohols, acetonitrile or their mixtures. The solvents beyond this range may damage or destroy this coated CSP.

The main innovation of immobilized supports such as CHIRALPAK© IB is the unprecedented possibility of using an extended series of solvents as mobile phase components and/or sample solvents without compromising the CSP stability. Similar to CHIRALPAK© IA, CHIRALPAK© IB can be used with all types of miscible organic solvents, progressing from the standard mobile phases compatible with the coated-type polysaccharide-derived CSPs to mobile phases containing chloroform (CHC3), ethyl acetate (EA), tetrahydrofuran (THF), MtBE and toluene, among others.

The potion to use the "non-standard" solvents in the mobile phase opens up new possibilities for unique selectivities. There are also no limitations on the sample injection solvent with CHIRALPAK© IB. Solvents such as dichloromethane, acetone, THF, dimethylformamide (DMF) or even dimethylsulfoxide (DMSO) can be safely and effectively used as sample diluents. This is highly beneficial for the automation of injections for samples coming directly from various synthetic media.

Method development on CHIRALPAK®IB

1. Mobile phase nature and enantioselectivity

The mobile phase is one of the key factors for a successful chiral separation. A series usual HPLC solvents and their mixutes have been examined in our laboratory. Two groups of solvents can be distinguished for CHIRALPAK© IB in terms of enantioselectivity.

Group 1	Group 2
► CHCl3	► toluene
► EA	► CHCl2
► THF	► acetone
► MtBE	► dioxane
► Alcohol in alkane	► acetonitrile

The solvents of the first group are those with the highest potential. Their presence in the mobile phase will usually lead to better enantioselectivities than solvents from Group 2. In general terms, we would recommend that you first try chloroform, THF, ethyl acetate, Mtbe or alcohols (preferably in combination with an alkane) on CHIRALPAK© IB for the reason of enatioselectivity.

Table 1 summarizes the separations of racemic laudanosine on CHIRALPAK© IB using various solvents from Group 1, and the best separation is shown in Figure 2.

Table 1. separations of laudanosine on CHIRALPAK©IB

Mobile phase		k'1	a	Rs
MtBE/EtOH/EDA*	95/5/0.1	0.64	3.77	14.47
n-hexane/THF/EDA	70/30/0.1	0.67	2.68	15.41
n-hexane/EA/EtNA*	70/30/0.05	2.10	2.14	17.59
n-hexane/CHCl3/EtNA	65/35/0.1	1.32	1.28	5.75
n-hexane/2-propanol/EDA	80/20/0.1	1.33	2.76	14.62

**EDA :ethylenediamine*
**EtAN : ethanolamine*

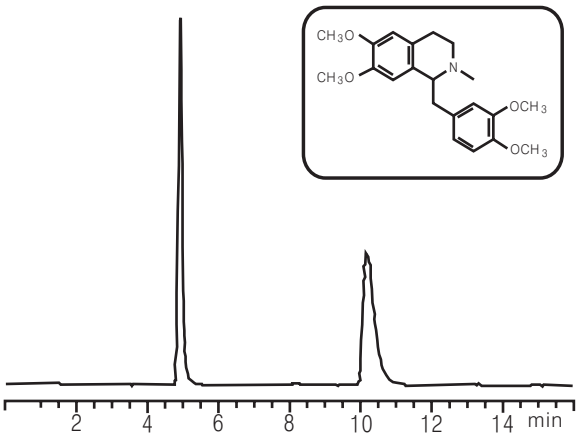


Figure 2. Separation of laudanosine on CHIRALPAK©IB

MtBE/EtOH/EDA 95/5/0.1 v/v/v ; 1.0 ml/min. 25℃
UV detection : 270nm.

Table 2. Typical non−=standard mobile phase compostions for CHIRALPAK©IB

	First choice				Second choice		
Non-standard solvent (NSS)	CHCl3	MtBE	EA	THF	CH2Cl2	Toluene	Acetone
Standard solvents(SS)	Alkane	Ethanol	Alkane	Alkane	Alkane	Alkane	Alkane
Typical starting conditions (NSS/SS))	50:50	98:2	40:60	30:70	40:60	70:30	25:75
Advised optimisation range	25:75 to 100:0	80:20 to 100:0	20:80 to 70:30	10:90 to 50:50	20:80 to 100:0	30:70 to 100:0	10:90 to 50:50

The typical non-standard mobile phase compositions for CHIRALPAK© IB are summarized in Table 2. For the use of standard mobile phases on CHIRALPAK©IB, please refer to our instruction manual for CHIRALCEL©OD.

The enantioresolution of many compounds can be achieved on CHIRALPAK© IB owing to the addition of chloroform to the mobile phase. Screenings using chloroform could be started with a 50/50 mixture in an alkane. However, 100% chloroform can be used for compounds that have strong interactions with the CSP. In some cases, the addition of a low percentage of alcohol (e.g. MeOH) may be required to elute the solutes within a reasonable time range.

MtBE can be used undiluted as a mobile phase. However, due to its relatively low eluting strength, the addition fo 2% alcohol (e.g. EtOH) may be helpful to shorten the retention time, improve the peak shape and enhance the resolution. In addition, Methanol, THF, acetone or dioxane may also be used as modifiers in MtBE and can somdtimes produce significant improvements of the separation.

Ethyl acetate and THF are stronger eluting solvents, and therefore should be kept at lower percentages in the mobile phase: 40% ethyl acetate in alkane and 30% THF in alkane to start the method development. The use of 70% ethyl acetate or 50% THF in mobile phases in still possible for some samples strongly retained on CHIRALPAK©IB. 100% THF or ethyl acetate often leads to no retention of solutes.

It should be noted that, if only mobile phases from the standard category are considered, CHIRALCEL© OD-H (the coated CSP having the same chiral selector) often shows better enantioselectivity than CHIRALPAK© IB. However, the option to use the non-standard solvents in the mobile phase allows enhancement of chiral separations in terms of enantioselectivity, resolution degree and efficiency.

Some separation examples on CHIRALPAK© IB using various mobile phase systems are shown on page 4.

2. Mobile phase additives

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

Among the basic additives listed in table 3, ethylenediamine (EDA) in the most efficient, followed by ethanolamine (EtNA), n-butylamine (BuA) and diethylamine (DEA). The addition of a low percentage of an alcohol (e.g. EtOH 2-5%) in the mobile phase may be helpful to ensure the miscibility of amine additives with the mobile phases of low polarity.

Table 3. Mobile phase additives

Basic samples require basic additives	Acidic samples require Acidic additives
ethylenediamine(EDA) n-butylamine(BuA) ethanolamine(EtNA) diethylamine(DEA)	TFA CH_3COOH HCOOH
<0.5% by volume Typically 0.1%	<0.5% by volume Typically 0.1%

Column cleaning adn regeneration procedures

Even though the chiral recognition mechanism on polysaccharide-derived CSPs has not been fully explained, it is a well-known hypothesis that their chiral separation ability depends to a certain extent upon the supramolecular structure of the polymeric chiral selector. It may be postulated that the immobilized polymer changes its supramolecular structure by adopting its conformation to the solvating environment. Fortunately, any changes in the CSP are reversible.

In order to ensure consistent performance after extensive use with different mobile phases, a regeneration method may be Necessary to eliminate any unexpected change of chiral recognition due to the mobile phase history of the column:

• Flush the column with ethyl acetate at 0.5 ml/min. for 30 min (> 2 hours if some additives are used in the previous mobile phases);

• Store the column at room temperature for 2 days or longer;

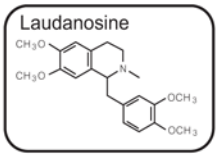
• Flush with hexane/IPA 90/10 v/v at 1.0 ml/min. for hour prior to retest the column.

CHIRALPAK© IB is another milestone in the immobilized series of polysaccharide-derived CSPs. Its solvent versatility, specific enantioselective performance and robustness make it a useful separating material in analytical and semi-preparative scales.

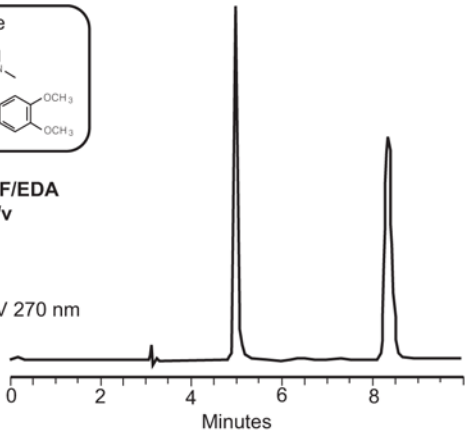
CHIRALPAK® IB

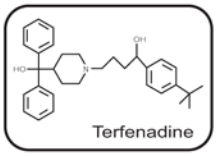
Analytical HPLC applications

Laudanosine

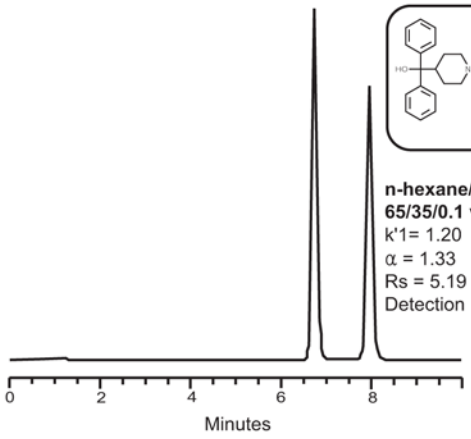


n-hexane/THF/EDA
70/30/0.1 v/v/v
k'1=0.67
α=2.68
Rs=15.41
Detection : UV 270 nm

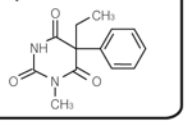




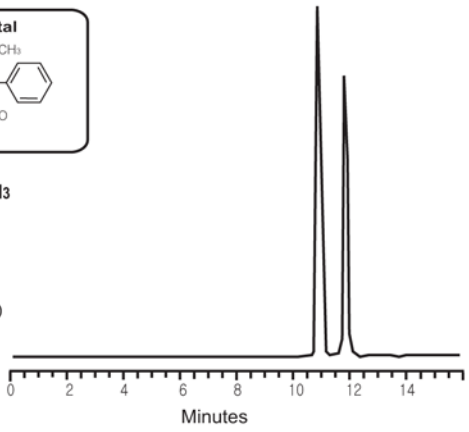
n-hexane/CHCl3/EtNA
65/35/0.1 v/v/v
k'1= 1.20
α = 1.33
Rs = 5.19
Detection : ELSD

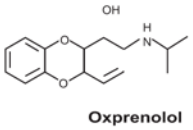


Mephobarbital

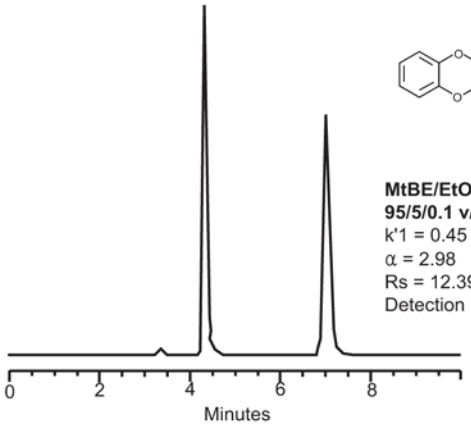


n-hexane/CHCl3
50/50 v/v
k'=2.44
α=1.12
Rs=3.68
Detection:ELSD

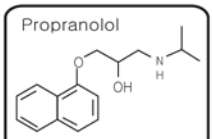




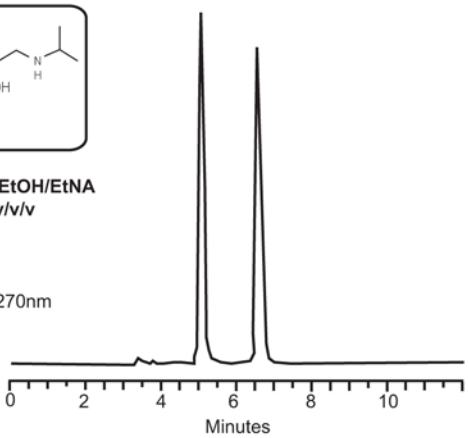
MtBE/EtOH/EDA
95/5/0.1 v/v/v
k'1 = 0.45
α = 2.98
Rs = 12.39
Detection : UV 270 nm

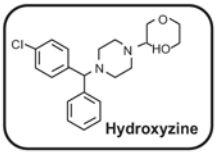


Propranolol

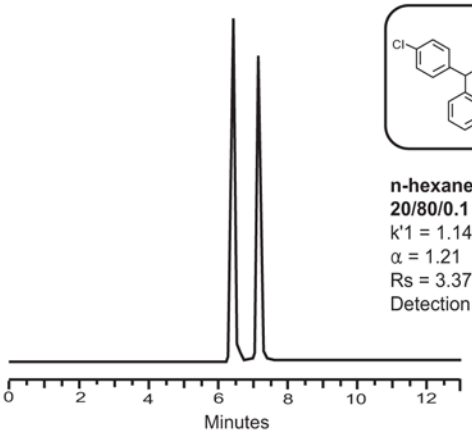


n-hexane/EA/EtOH/EtNA
50/50/2/0.1 v/v/v/v
k'1=0.69
α=1.74
Rs=6.49
Detection:UV 270nm





n-hexane/toluene/EDA
20/80/0.1 v/v/v
k'1 = 1.14
α = 1.21
Rs = 3.37
Detection : ELSD



General conditions: CHIRALPAK® IB 25 X 0.46cm, Flow rate: 1 ml/min, 25℃

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