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Pure simplicity

CHIRALPAK® IB: A new Daicel column combining high performance and solvent versatility for the separation of chiral compounds

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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.

Introduction

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 product 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.

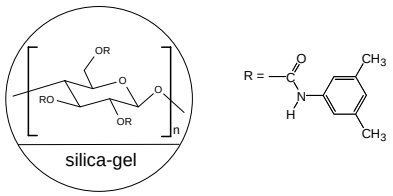

Figure 1. Packing composition of CHIRALPAK® IB

This document focuses predominantly on chromatographic method development and optimisation of chiral separation with CHIRALPAK® IB. In addition its main features, its complementary character to CHIRALPAK® IA and CSP stability will also be discussed.

Main features 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 "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 ($CHCl_3$), ethyl acetate (EA), tetrahydrofuran (THF), MtBE and toluene, among others.

The option 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 dimethyl sulfoxide (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.

Reversal of elution order of some enantiomers may be observed with different mobile phases. This feature may be beneficial when the determination of the enantiomeric excess of an enriched sample or a preparation in a semi-preparative scale of a specially desired enantiomer is needed.

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 of usual HPLC solvents and their mixtures have been examined in our laboratory. Two groups of solvents can be distinguished for CHIRALPAK® IB in terms of enantioselectivity.

Group 1	Group 2
$CHCl_3$	toluene
EA	CH_2Cl_2
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 enantioselectivity.

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	α	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/$CHCl_3$/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* ** *EtNA: ethanolamine*

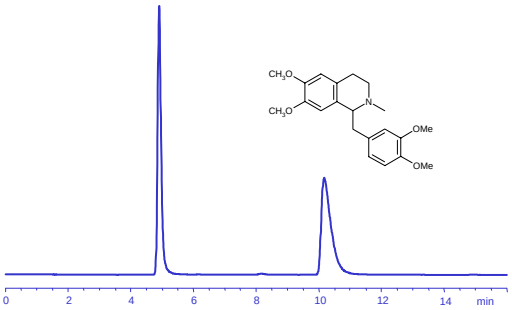

Figure 2. Separation of laudanosine on CHIRALPAK® IB
MtBE/EtOH/EDA 95/5/0.1 v/v/v ; 1.0 ml/min. 25°C
UV detection: 270nm.

To assist users with some typical starting conditions and ranges for method development, we would like to propose the following guidelines. 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 with 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 2-5%) may be required to elute the solutes within a reasonable time range. Compared with chloroform, dichloromethane has stronger eluting strength and generally leads to lower enantioselectivity with CHIRALPAK® IB.

MtBE can be used undiluted as a mobile phase. However, due to its relatively low eluting strength, the addition of 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 sometimes 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 is still possible for some samples strongly retained on CHIRALPAK® IB. 100% THF or ethyl acetate often leads to no retention of solutes.

Some separation examples on CHIRALPAK® IB using various mobile phase systems are shown in Figures 2.

Table 2. Typical non-standard mobile phase compositions for CHIRALPAK® IB

	First choice				Second choice		
Non-standard solvent (NSS)	$CHCl_3$	MtBE	EA	THF	CH_2Cl_2	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

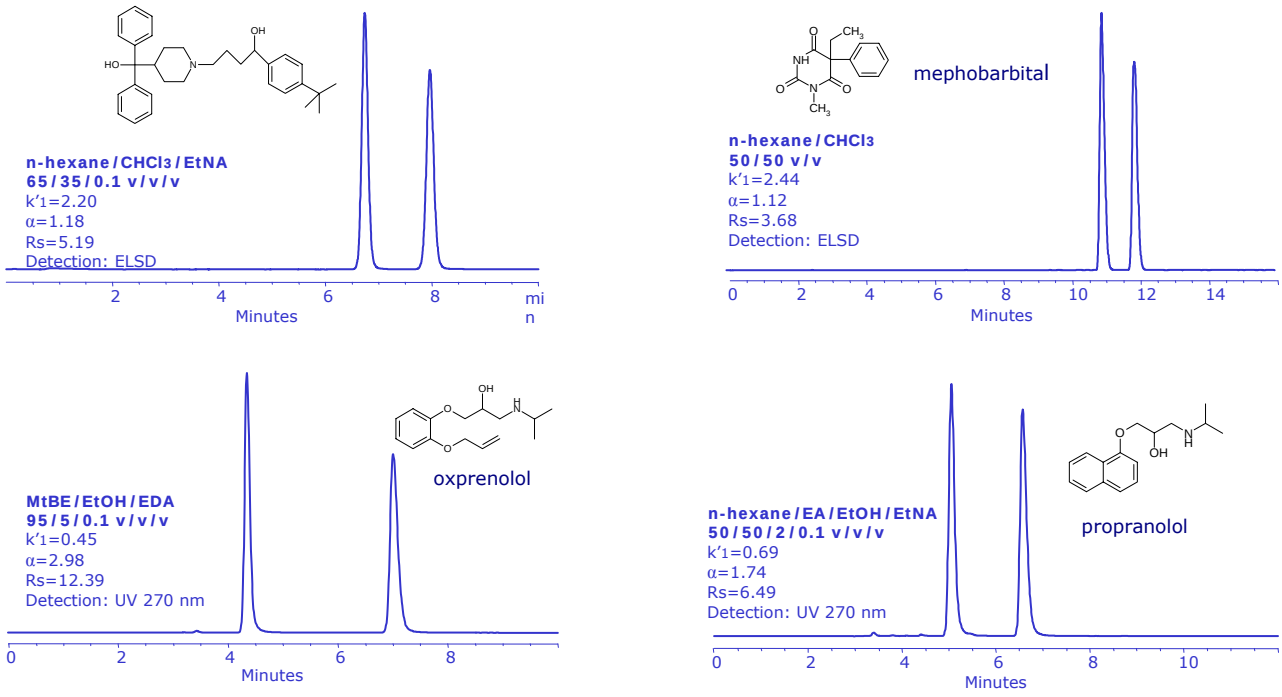

Figure 2. Separation examples on CHIRALPAK® IB using various mobile phases *(Column 250 x 4.6 mm ID, 25°C, 1.0 mL/min.)*

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. The first chromatogram in Figure 2 shows a better separation of terfenadine on CHIRALPAK® IB than can be obtained with CHIRALCEL® OD-H.

It is well known that a chiral separation on a given CSP may be compound and eluent specific. If no satisfactory separation is found after the screening of the solvents in Group 1, it may be helpful to try the solvents in Group 2, such as toluene, acetone and dioxane. Figure 3 shows a separation of hydroxyzine with toluene as the major component of the mobile phase.

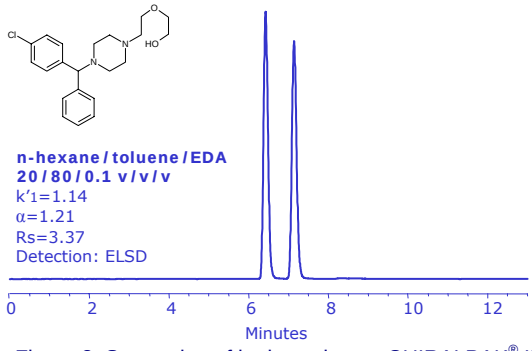

Figure 3. Separation of hydroxyzine on CHIRALPAK® IB
(Column 250 x 4.6 mm ID, 25°C, 1.0 mL/min.)

UV detection may become defective when solutes have weak absorption bands in UV or solvents have high UV cut-off (e.g. toluene and acetone). In this case, an alternative detector such as ELSD (Evaporative Light Scattering Detector, available now for a similar price to that of a UV/Vis detector) may be effective for the detection.

2. Mobile phase additives

It is often necessary to incorporate an additive into the mobile phase for the chiral separation of basic or acidic samples. In general, acidic samples require an acidic additive (TFA, acetic acid, formic acid, etc.), and compounds of basic nature may require the addition of a basic component. The percentage needed is typically 0.1% and should not exceed 0.5%.

For basic compounds, the nature of the basic additive may have a profound influence on their separation on CHIRALPAK® IB. Four commonly used basic additives were examined in our laboratory:

- ethylenediamine (EDA)
- n-butylamine (BuA)
- ethanolamine (EtNA)
- diethylamine (DEA)

The effectiveness of these basic additives on CHIRALPAK® IB is in the following order:

EDA > EtNA > BuA > DEA

EDA is the strongest and most effective. Not only can it induce the highest resolution degree and the best peak symmetry, it also reduces significantly the retention time without deteriorating the selectivity of the chiral separations. The separation of racemic propranolol in Figure 4 with an n-hexane/2-propanol 80/20 v/v based mobile phase demonstrates this point.

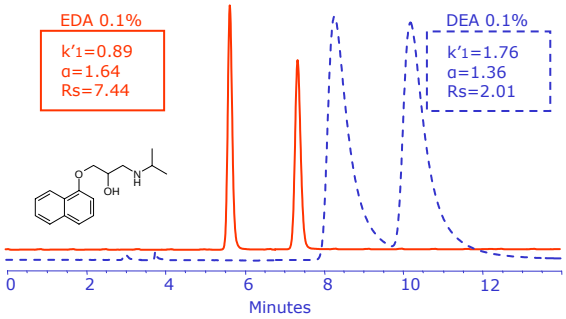

Figure 4. Chiral resolution of propranolol on CHIRALPAK® IB
(Column 250 x 4.6 mm ID, 25°C, 1.0 mL/min.)

We recommend the use of EDA as mobile phase additive for separations of basic compounds on CHIRALPAK® IB. However, it should be noted that the addition of an alcohol at low percentage (e.g. EtOH 2-5%) is sometimes necessary to ensure the miscibility of amine additives with mobile phases of low polarity.

Complementary properties of CHIRALPAK® IB to CHIRALPAK® IA

Due to their different polysaccharide natures, CHIRALPAK® IA and CHIRALPAK® IB have their own unique selectivity characteristics and, at the same time, offer complementary separations in many cases.

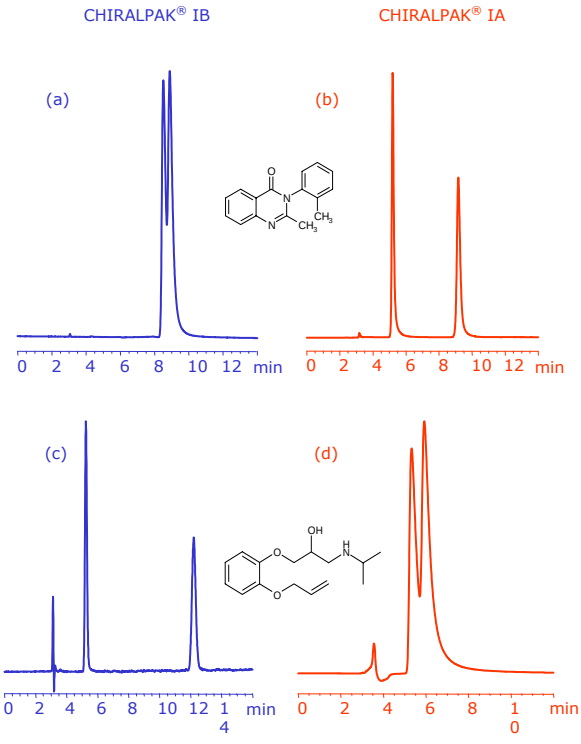

Figure 5. Separation of methaqualone (a-b) and oxprenolol (c-d) on CHIRALPAK® IB and CHIRALPAK® IA
(a). n-hexane/EA 70/30 v/v; (b). MtBE/EtOH 95/5 v/v;
(c). n-hexane/THF/EtNA 70/30/0.1 v/v/v; (d). MtBE/DEA 100/0.1 v/v
(Column 250 x 4.6 mm ID, 25°C, 1.0 mL/min).

Methaqualone is only partially resolved on CHIRALPAK® IB (Figure 5a) with n-hexane/EA 70/30. However, the same compound is well resolved on CHIRALPAK® IA if a mixture of MtBE/EtOH 95/5 is used as the mobile phase (Figure 5b). Inversely, racemic oxprenolol can barely be resolved on CHIRALPAK® IA (Figure 5c), but a large separation can easily be obtained (Figure 5d) with n-hexane/THF/EtNA 70/30/0.1 v/v/v on CHIRALPAK® IB.

Column regeneration of CHIRALPAK® IB

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.

The following is our proposed CSP regeneration method for CHIRALPAK® IB columns:

- 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 1 hour prior to retest the column.

Such a column treatment should recover the initial conformation adopted by the polymer. The insertion of this regeneration sequence after intensive changes between very different solvent mixtures allows the user to achieve reproducible chromatographic results.

Stability of the CSP

As previously mentioned, the coated polymer (in CHIRALCEL® OD, for example) can be stripped off if a non-standard mobile phase is used. However, CHIRALPAK® IB is considered to be resistant to all kinds of mobile phases.

The mixture of $CHCl_3$/MeOH 98/2 v/v can readily lead to a total dissolution of the chiral polymer cellulose tris(3,5-dimethyl phenylcarbamate) in its pure form. In order to examine the stability of the CHIRALPAK® IB CSP, this mixture was used as the mobile phase to repeat the injection of oxazepam on a CHIRALPAK® IB column. 400 injections of oxazepam were repeated within a time length of 80 hours. Figure 9 shows the overlaid chromatograms from the first and the 400th injections of oxazepam.

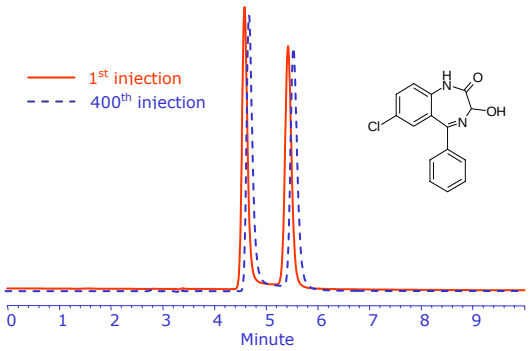

Figure 9. 1st injection and 400th injections of oxazepam
$CHCl_3$/MeOH 98/2 v/v; 1.0ml/min., 25°C

The satisfactory repeatability between the first injection and the 400th injection proves that the polymer is completely immobilized on the matrix and the CHIRALPAK® IB CSP is totally stable.

Outlook

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 chiral separating material in analytical and semi-preparative scales.

The market introduction of CHIRALCEL® OD-I, the immobilized version of 20 µm CHIRALCEL® OD, before the end 2004 and the bulk 20µm CHIRALPAK® IA material in Spring 2005 will undoubtedly enforce the industrial applications of chiral chromatography in continuous processing mode, such as Simulated Moving Bed (SMB).

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