Performance Evaluation of Immobilized and Coated Polysaccharide Chiral HPLC Columns Using Generic Screening Mobile Phase Systems

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Two chiral stationary phases (Lux® Cellulose-1 and CHIRALPAK®IB) that consist of the same chiral selector, cellulose tris(3,5-dimethylphenylcarbamate), were evaluated. The major difference between the two phases is that the first phase (Lux Cellulose-1) is prepared by coating the underlying silica with the modified polysaccharide, while the second phase is an immobilized phase in which the polysaccharide is covalently bonded to the underlying silica. This covalent linkage allows for the use of an extended range of solvents (e.g., THF, DMF, acetone, ethyl acetate, methylene chloride) that are not compatible with coated chiral stationary phases (CSPs). In this study, we sought to determine if this expanded solvent range increased the success rate of chiral separations using the immobilized CSP.

Introduction

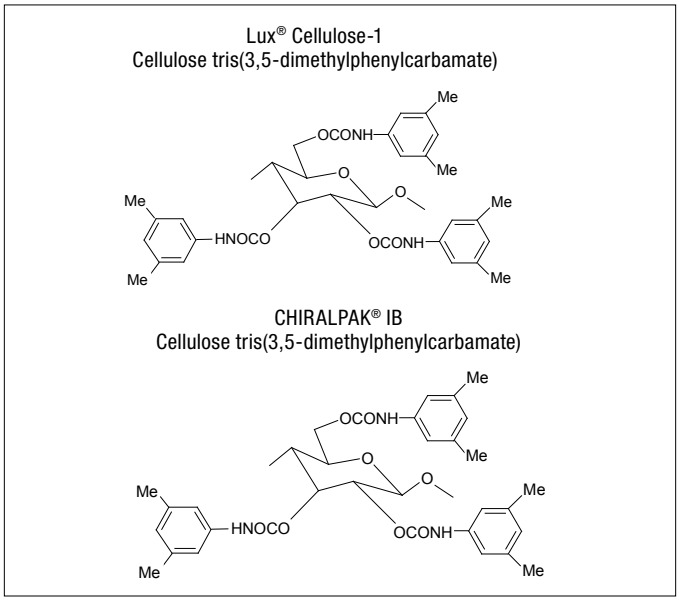
Modified polysaccharide-based stationary phases are the most widely used CSPs due to their broad-spectrum chiral selectivity and high loading capacity. Most separations performed using polysaccharide CSPs are performed in normal phase using solvents such as hexane and alcohol, and these conditions have been proven to be very favorable for chiral recognition mechanisms.

The majority of polysaccharide-based CSPs are coated phases, in which the stationary phase is not covalently bonded to the underlying silica. Recently, immobilized polysaccharide CSPs, in which the polysaccharides are covalently linked to the silica, have also become available. Immobilized CSPs allow for the use of more aggressive solvents, such as chlorinated solvents (e.g., methylene chloride) or ethyl acetate, which cannot be used with conventional coated phases due to solubility issues.

There has been speculation that the expanded solvent range of immobilized CSPs might increase the selectivity options and, hence, lead to enhanced enantiorecognition relative to coated CSPs. However, there is a lack of extensive comparative studies to determine if this is indeed the case. Thus, in this study, we have sought to determine if an immobilized CSP (CHIRALPAK IB) exhibits significantly increased chiral separation success rates as compared to a coated CSP (Lux Cellulose-1), when evaluated under generic normal phase screening conditions (including the use of chlorinated solvents). The two chiral stationary phases evaluated in this study consist of the same chiral selector, cellulose tris(3,5-dimethylphenylcarbamate) as depicted in **Figure 1**.

Generic screens in Normal Phase (NP) are common in the industry, as NP is favorable for the principal mechanisms of chiral recognition. The majority of chiral separations with polysaccharide phases are performed using hexane and alcohol modifiers. Previous work has identified the different selectivities offered between Isopropyl alcohol (IPA) and Ethanol (EtOH), and we have used these solvents in our generic screen of both columns. Chlorinated solvents and ethyl acetate may also be used as NP modifiers to offer a different analyte solvent selectivity; however, these cannot be used on coated polysaccharide columns. Our generic screen incorporated the addition of the above solvents only for the immobilized phase.

Figure 1.
Structures of cellulose based chiral stationary phase used in this study



Material and Methods

All analyses were performed using an HPLC Agilent® 1100 series (Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler and a quaternary pump. Chiral chromatographic separations followed by UV detection were performed using Lux Cellulose-1 (coated phase) and CHIRALPAK® IB (immobilized phase) HPLC columns with dimensions 250 x 4.6 mm ID packed with 5 µm particles. The system flow rate was set to 1 mL/min and the column temperature was ambient unless noted otherwise Mobile Phase Conditions used for each column are described in **Table 1**.

Table 1.
Mobile phase conditions used in this study.

CSP	Mobile Phase (MP)
Coated Phase Lux® Cellulose-1	80:20:0.1 Hex:IPA:DEA; and 85:15:0.1 Hex:EtOH:DEA
Immobilized Phase CHIRALPAK® IB	80:20:0.1 Hex:IPA:DEA; 85:15:0.1 Hex:EtOH:DEA; 65:35:0.1 Hex:EtOAc:Ethanolamine; and 65:35:0.1 Hex:$CHCl_3$:Ethanolamine

DEA = Diethyl amine; IPA = Isopropyl alcohol; $CHCl_3$ = Chloroform; Hex = Hexane; EtOH = Ethanol; EtOAc = Ethyl acetate

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Results and Discussion

To evaluate the enantioresolution between coated and immobilized CSP, 51 chemical compounds of pharmaceutical interest were analyzed under various mobile phase conditions. In the first set of experiments, IPA was used as a modifier with DEA as additive. Out of those 51 compounds, 22 were resolved on either CSPs using 80:20:0.1 Hexane:IPA:DEA as mobile phase. **Table 2** summarizes the difference in enantioselectivity using those conditions.

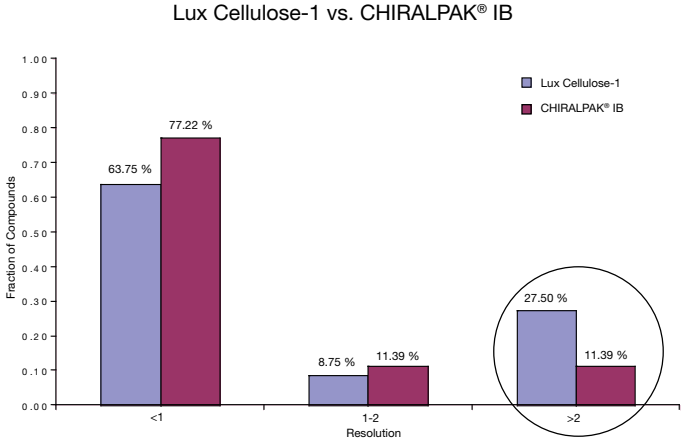
Table 2.
Comparison of enantioresolution of 22 racemates between coated and immobilized CSP using 80:70:0.1 Hex:IPA:DEA as organic modifier

Compounds	Lux® Cellulose-1	CHIRALPAK® IB	Compounds	Lux Cellulose-1	CHIRALPAK® IB
Tetrahydrozoline	√	x	Toliprolol	√	√
Metoprolol	√	x	Bisoprolol	√	Partial
Tetramisole	√	x	Sulfconazole	x	Partial
Halofantrine	√	√	Orphenadrine	√	√
Bopindolol	√	Partial	Mianserin	√	x
Bupranolol	√	Partial	1,1-Dihydroxy-6,6-Dimethylbiphenyl	√	√
Carazolol	Partial	x	Methoxy-p-tolyl sulfoxide	√	x
Metomidate	√	x	Prilocaine	√	Partial
Mephenesin	√	x	Nifedpine	x	√
Oxazapam	√	√	Bupivacaine	√	x
Oxprenolol	x	√	Disopyramide	Partial	x

	Lux Cellulose-1	CHIRALPAK® IB
√ Baseline Resolution $R_s > 1.5$	17	7
x Partial Resolution $0.8 < R_s < 1.5$	2	5
No resolution $R_s < 0.8$	3	10

The percentage of compounds that showed a resolution > 2 on coated Lux Cellulose-1 is over twice that observed on immobilized CHIRALPAK® IB using Hexane/IPA as mobile phase as represented in **Figure 2**.

Figure 2.
Percentage of compounds showing resolution > 2 using IPA as modifier.



Flow Rate: 1.0 mL/min
Temperature: 25 °C
Detection: 220 nm
Mass Loaded: 10 µg

The same 51 compounds were analyzed under different mobile phase conditions using EtOH as organic modifier with DEA as additive. Out of those 51 compounds, 28 analytes were resolved on either CSP using 85:15:0.1 Hexane:EtOH:DEA as mobile phase. **Table 3** summarizes the difference in enantioselectivity using those conditions.

Table 3.
Comparison of enantioresolution of 28 racemates between coated and Immobilized CSP using 85:15:0.1 Hex EtOH:DEA as organic modifier

Compounds	Lux® Cellulose-1	CHIRALPAK® IB	Compounds	Lux Cellulose-1	CHIRALPAK® IB
DL-B-Hydroxyphenethylamine	x	Partial	Carazolol	√	Partial
Miconazole	√	Partial	Toliprolol	√	√
Tetrahydrozoline	√	x	Bisoprolol	√	Partial
Metoprolol	x	√	Sulfconazole	√	√
Acebutolol	Partial	x	Orphenadrine	√	Partial
Tetramisole	√	√	Mianserin	√	Partial
Halofantrine	√	√	1,1-Dihydroxy-6,6-Dimethylbiphenyl	Partial	√
Bopindolol	√	√	Methoxy-p-tolyl sulfoxide	√	x
Bupranolol	√	x	5-Methyl-5-phenyl-hydantoin	Partial	x
Metomidate	√	Partial	Nifedpine	x	√
Mephenesin	√	Partial	Bupivacaine	Partial	x
Oxazapam	√	√	Omeprazole	√	√
Oxprenolol	√	√	Indapamide	x	√
Prilocaine	Partial	Partial	Bendroflumethiazide	x	√

	Lux Cellulose-1	CHIRALPAK® IB
√ Baseline Resolution $R_s > 1.5$	18	13
x Partial Resolution $0.8 < R_s < 1.5$	5	9
No resolution $R_s < 0.8$	5	6

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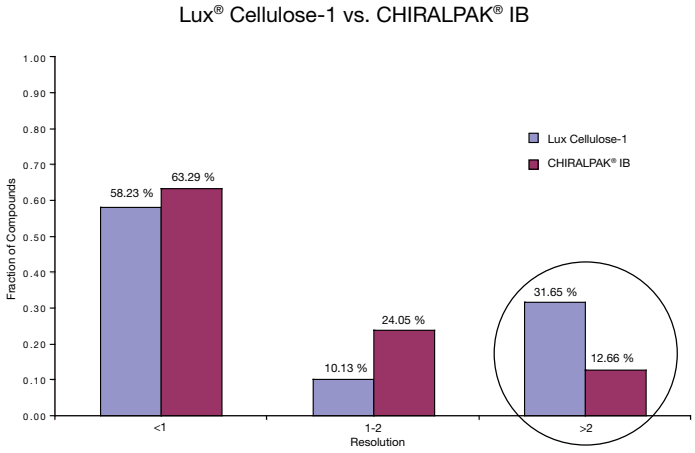
The percentage of compounds that showed a resolution > 2 on coated Lux® Cellulose-1 is over twice that observed on immobilized CHIRALPAK IB using Hexane/EtOH as mobile phase as represented in **Figure 3**.

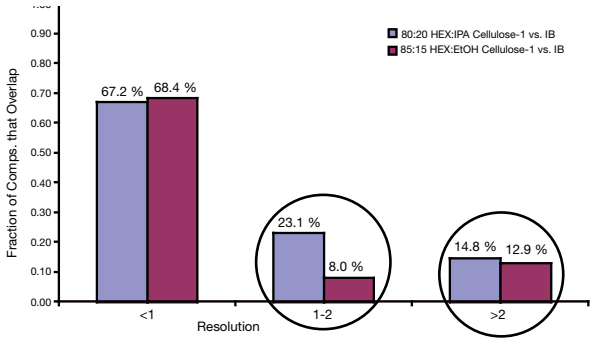
Figure 3.
Percentage of compounds showing resolution > 2 using EtOH as modifier



Flow Rate: 1.0 mL/min
Temperature: 25 °C
Detection: 220 nm
Mass Loaded: 10 µg

Interestingly, although both columns shared similar chiral selector, cellulose tris(3,5-dimethylphenylcarbamate), there was very little correlation between the compounds that were resolved on the coated phase and those that were resolved on the immobilized phase (< 25 % of the racemates were resolved on both columns under identical running conditions) as depicted in **Figure 4**.

Figure 4.
Extent of Complementary Selectivity between Coated and Immobilized CSPs in Hex:IPA and Hex:EtOH



% Match of Compounds Resolved on both Lux Cellulose-1 and CHIRALPAK IB for Different Resolution Ranges

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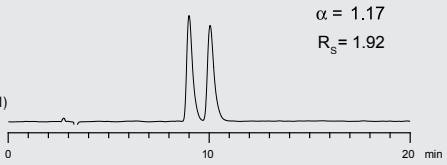
A few representative examples of the effect on the mobile phase composition on the chiral separation on coated and immobilized CSP are presented on **Figure 5.** The data clearly indicates that under the same mobile phase conditions, coated CSP Lux Cellulose-1 has a greater separation properties over immobilized CSP.

Figure 5.
Effect of Organic Modifier on Lux Cellulose-1 (coated) and CHIRALPAK® IB (immobilized) CSPs

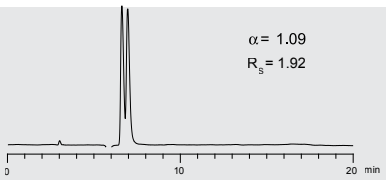
Mephenesin

Columns: Lux® 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



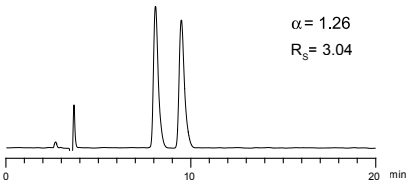
App ID 19195

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



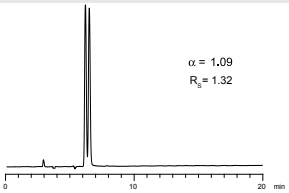
App ID 19196

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19197

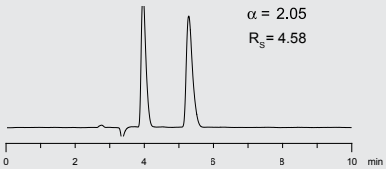
Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19198

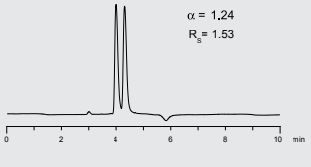
Orphenedrine®

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



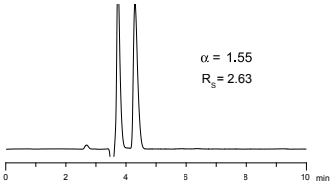
App ID 19199

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



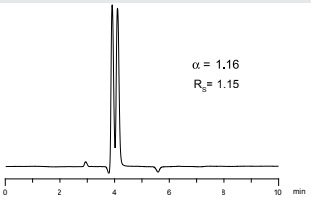
App ID 19200

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19201

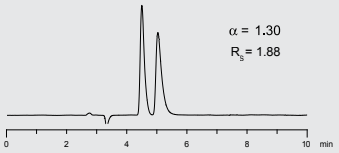
Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19202

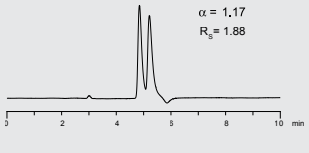
Prilocaine

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



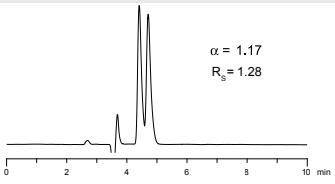
App ID 19203

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



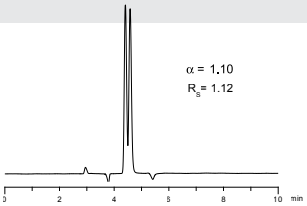
App ID 19204

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19205

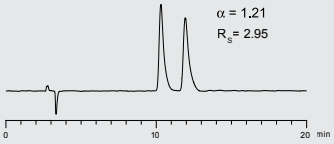
Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19206

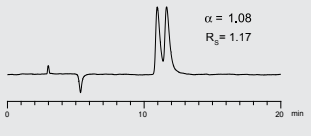
Tetramisole

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Heptane/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



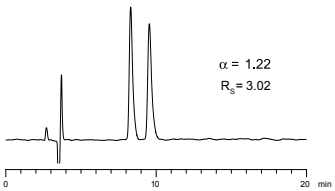
App ID 19207

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Heptane/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



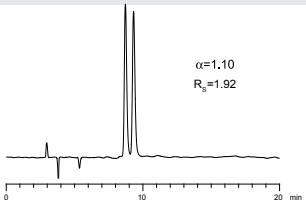
App ID 19208

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19209

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm

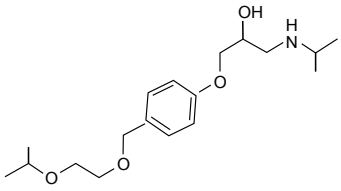


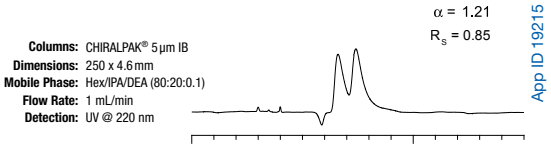
App ID 19210

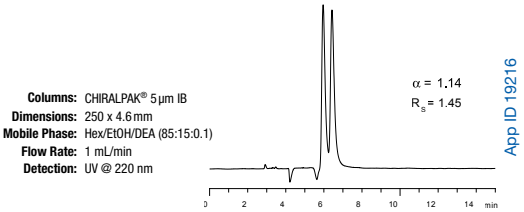
APPLICATIONS

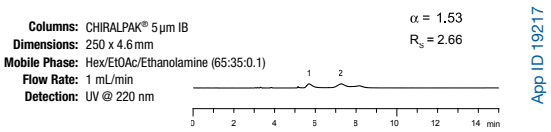
Immobilized CSP can be used with mobile phases of various natures, ranging from the so called “standard solvents “ such as acetonitrile, alcohols, and alkanes recommended for coated CSPs to mobile phase containing “non-standard” solvents such as chlorinated solvents, ethyl acetate, tetrahydrofuran (THF) and methyl tertiary butyl ether (MTBE). **Figures 6-8** show separation of three chiral analytes using “standard” and “non-standard” solvents indicating that using chloroform ($CHCl_3$) or ethyl acetate (EtOAc) in the mobile phase does not necessarily improve the chiral separation.

Figure 6.
Bisoprolol on CHIRALPAK® IB









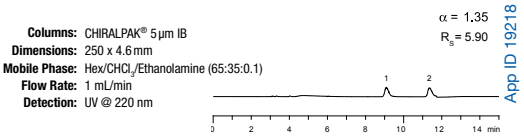
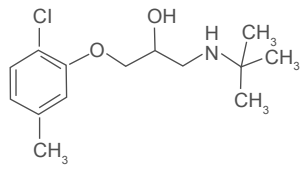
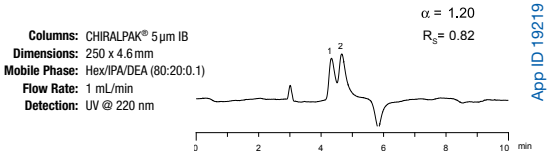
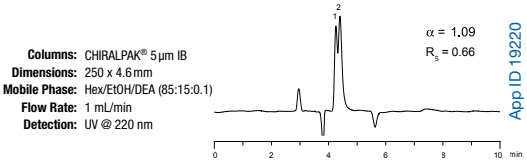
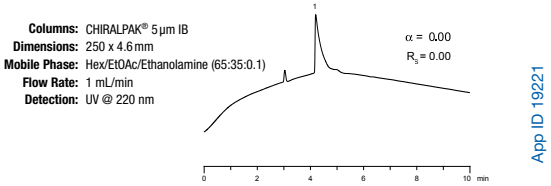


Figure 7.
Bupranolol on CHIRALPAK® IB









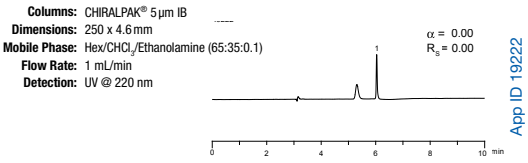
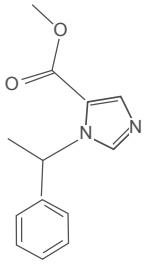
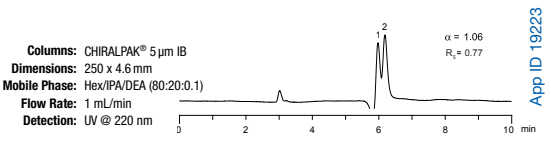
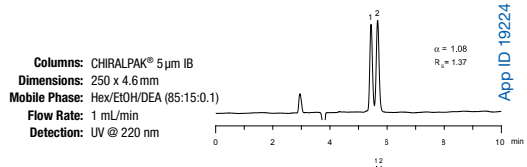
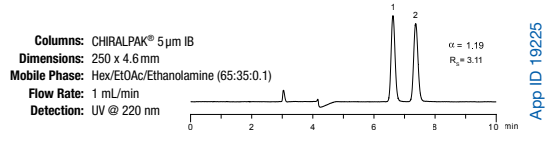


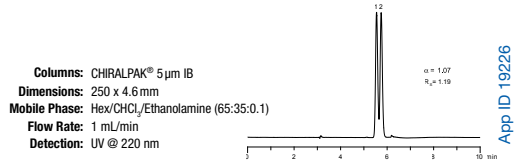
Figure 8.
Metomidate on CHIRALPAK® IB











APPLICATIONS

Of the 51 unique compounds investigated using various NP solvent systems only 18 were partially or fully resolved using Chiralpak IB as summarized in **Table 4**. The option of using non-standard solvents on immobilized CSPs clearly did not offer any advantages over coated CSP for those 51 compounds.

Table 4.

Compounds	80:20:0.1 Hex:IPA:DEA	85:15:0.1 Hex:EtOH:DEA	65:35:0.1 Hex:EtOAc:Ethanolamine	65:35:0.1 Hex:$CHCl_3$:Ethanolamine
1,1-Dihydroxy-6,6-Dimethylbiphenyl	√	√	x	Partial
Bisoprolol	Partial	Partial	√	√
Bopindolol	Partial	√	x	√
Bupranolol	Partial	x	x	√
Disopyramide	x	x	√	x
Mephenesin	Partial	Partial	√	x
Methoxy-p-tolyl sulfoxide	x	x	Partial	x
Metomidate	x	Partial	√	Partial
Metoprolol	x	√	√	x
Mianserin	x	Partial	Partial	Partial
Miconazole	x	Partial	Partial	x
Orphenadrine	√	Partial	√	√
Oxprenolol	√	√	√	√
Prilocaine	Partial	Partial	√	√
Sulfconazole	√	√	√	√
Tetramisole	Partial	√	Partial	x
Toliprolol	√	√	√	x
Zopiclone	x	x	√	√

√ Baseline resolution: $R_s > 1.5$
x No resolution $R_s < 0.8$
Partial resolution $0.8 < R_s < 1.5$

Conclusions

In this Technical Note, we compared the chiral® separation success rate of an immobilized CSP (CHIRALPAK IB) to a conventional coated CSP (Lux® Cellulose-1) under generic normal phase screening conditions using 51 different racemates of pharmaceutical interest.

Using the conventional mobile phase of Hexane/IPA/DEA, the coated Lux Cellulose-1 column was able to resolve 17 racemates, while the immobilized CHIRALPAK® IB column was only able to resolve 7 racemates. Using another conventional mobile phase (Hexane/Ethanol/DEA), the coated phase column was able to resolve 18 racemates, while the immobilized phase column was only able to resolve 13 racemates with baseline or greater resolution.

The overall resolution success rate of the coated Lux Cellulose-1 column was 45 % (Rs > 2) using two mobile phases (Hexane/IPA/DEA and Hexane/EtOH/DEA) compared to the overall success rate of 37 % for the immobilized CHIRALPAK IB columns using four different mobile phases (Hexane/IPA/DEA, Hexane/EtOH/DEA, Hexane/Chloroform/Ethanolamine, and Hexane/Ethyl acetate/Ethanolamine).

Overall, the data indicates that, under generic normal phase screening conditions, traditional coated CSPs display greater enantioselectivity in terms of % of compounds resolved with greater than baseline resolution than do immobilized CSPs.

APPLICATIONS

Lux® Ordering Information

3 µm Analytical Columns (mm)							SecurityGuard™ Cartridges (mm)	
Phases	50 x 2.0	150 x 2.0	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 2.0*	4 x 3.0*
Cellulose-1	00B-4458-B0	00F-4458-B0	00B-4458-E0	00D-4458-E0	00F-4458-E0	00G-4458-E0	AJ0-8402	AJ0-8403
Cellulose-2	00B-4456-B0	00F-4456-B0	00B-4456-E0	00D-4456-E0	00F-4456-E0	00G-4456-E0	AJ0-8398	AJ0-8366
Cellulose-3	00B-4492-B0	00F-4492-B0	00B-4492-E0	00D-4492-E0	00F-4492-E0	00G-4492-E0	AJ0-8621	AJ0-8622
Cellulose-4	00B-4490-B0	00F-4490-B0	00B-4490-E0	00D-4490-E0	00F-4490-E0	00G-4490-E0	AJ0-8626	AJ0-8627
Amylose-2	00B-4471-B0	00F-4471-B0	00B-4471-E0	00D-4471-E0	00F-4471-E0	00G-4471-E0	AJ0-8471	AJ0-8470
						for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm Analytical Columns (mm)						SecurityGuard Cartridges (mm)	
Phases	50 x 2.0	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 2.0*	4 x 3.0*
Cellulose-1	00B-4459-B0	00B-4459-E0	00D-4459-E0	00F-4459-E0	00G-4459-E0	AJ0-8402	AJ0-8403
Cellulose-2	00B-4457-B0	00B-4457-E0	00D-4457-E0	00F-4457-E0	00G-4457-E0	AJ0-8398	AJ0-8366
Cellulose-3	00B-4493-B0	00B-4493-E0	00D-4493-E0	00F-4493-E0	00G-4493-E0	AJ0-8621	AJ0-8622
Cellulose-4	00B-4491-B0	00B-4491-E0	00D-4491-E0	00F-4491-E0	00G-4491-E0	AJ0-8626	AJ0-8627
Amylose-2	00B-4472-B0	00B-4472-E0	00D-4472-E0	00F-4472-E0	00G-4472-E0	AJ0-8471	AJ0-8470
					for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm Semi-Prep Columns (mm)			SecurityGuard Cartridges (mm)
Phases	150 x 10.0	250 x 10.0	10 x 10.0‡
Cellulose-1	00F-4459-N0	00G-4459-N0	AJ0-8404
Cellulose-2	00F-4457-N0	00G-4457-N0	AJ0-8399
Cellulose-3	00F-4493-N0	00G-4493-N0	AJ0-8623
Cellulose-4	00F-4491-N0	00G-4491-N0	AJ0-8628
Amylose-2	00F-4472-N0	00G-4472-N0	AJ0-8472
		for ID:	9–16 mm

Bulk Media		
Phases	100 g	1 kg
10 µm		
Cellulose-1	04G-4501	04K-4501
Cellulose-2	04G-4502	04K-4502
20 µm		
Cellulose-1	04G-4473	04K-4473
Cellulose-2	04G-4464	04K-4464
Cellulose-3	04G-4504	04K-4504
Cellulose-4	04G-4503	04K-4503

Please inquire for 20 µm Lux Amylose-2 media

5 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard Cartridges (mm)	
Phases	150 x 21.2	250 x 21.2	250 x 30	250 x 50	15 x 21.2**	15 x 30.0♦
* Cellulose-1	00F-4459-P0-AX	00G-4459-P0-AX	00G-4459-U0-AX	00G-4459-V0-AX	AJ0-8405	AJ0-8406
* Cellulose-2	00F-4457-P0-AX	00G-4457-P0-AX	00G-4457-U0-AX	00G-4457-V0-AX	AJ0-8400	AJ0-8401
Cellulose-3	00F-4493-P0-AX	00G-4493-P0-AX	00G-4493-U0-AX	00G-4493-V0-AX	AJ0-8624	AJ0-8625
Cellulose-4	00F-4491-P0-AX	00G-4491-P0-AX	00G-4491-U0-AX	00G-4491-V0-AX	AJ0-8629	AJ0-8630
Amylose-2	00F-4472-P0-AX	00G-4472-P0-AX	00G-4472-U0-AX	00G-4472-V0-AX	AJ0-8473	AJ0-8474
*Inquire for cellulose 1 and 2 10 µm				for ID:	18–29 mm	30–49 mm

*SecurityGuard Analytical Cartridges require holder, Part No. : KJ0-4282
‡SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-7220
**PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8223
♦PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8277

guarantee

If Lux analytical columns (≤ 4.6 mm ID) do not provide at least an equivalent or better separation as compared to competing column with similar dimension, phase, and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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APPLICATIONS

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