TN-1047
APPLICATIONS

Alternative Selectivity of Chiral Stationary Phases Based on Cellulose tris(3-chloro-4-methylphenylcarbamate) and Cellulose tris(3,5-dimethylphenylcarbamate)

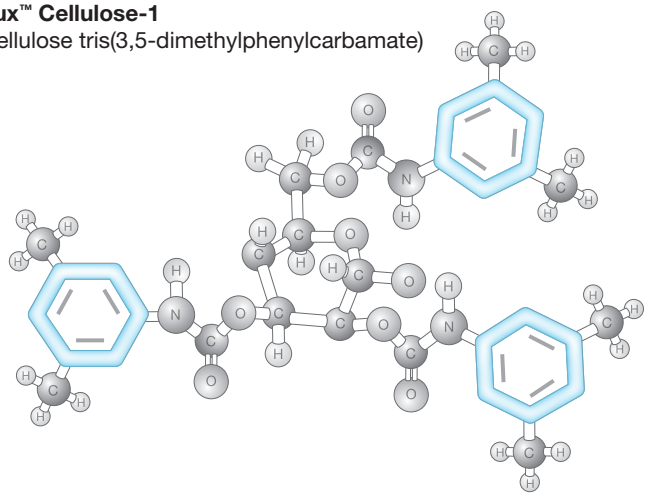
Liming Peng, Tivadar Farkas and Swapna Jayapalan
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

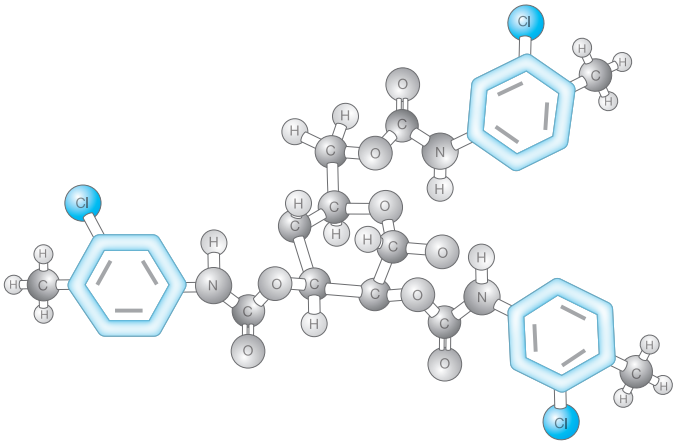
Polysaccharide-based chiral stationary phases (CSP) are widely used due to their wide chiral recognition ability. Several cellulose and amylose derivatives are extremely effective in the separation of a wide range of compounds of interest in the pharmaceutical industry[1]. This work demonstrates the different chiral recognition capabilities of CSPs based on cellulose tris(3-chloro-4-methylphenylcarbamate) and cellulose tris(3,5-dimethylphenylcarbamate). Over 180 racemates of pharmaceutical interest were analyzed on these two phases in normal (NP), polar-organic (PO) and reversed phase (RP) separation modes. Numerous examples including important classes of drug compounds as well as statistical data prove that cellulose tris(3-chloro-4 methylphenylcarbamate) offers a good alternative to the commonly used cellulose tris(3,5- dimethylphenylcarbamate) in the separation of difficult racemic mixtures.

Figure 1.
Structures of Chiral Selective Phases

Lux™ Cellulose-1
Cellulose tris(3,5-dimethylphenylcarbamate)



Lux™ Cellulose-2
Cellulose tris(3-chlorlo-4-methyl-dimethylphenylcarbamate)



HPLC Conditions

Instrumentation	
HPLC System:	Agilent 1100 series (www.agilent.com)
Pump:	G1311A Quaternary Pump
Autosampler:	G1313A ALS
Detector:	G1315A Diode Array Detector

HPLC Conditions	
Flow Rate:	1.0 mL/min
Injection Volume:	5 - 20 µL (depending on analyte response)
Sample Concentration:	500 µg/mL racemate dissolved in mobile phase
Columns:	Lux™ 5 µm Cellulose-1; 250 x 4.6 mm Lux™ 5 µm Cellulose-2; 250 x 4.6 mm CHIRALCEL® 5 µm OD-H®; 250 x 4.6 mm
Temperature:	Ambient
Detector:	UV @ 220 nm

Table 1.
Mobile Phase Compositions

Mobile Phase	NP	PO	
Basic and Neutral Compounds	0.1 % DEA in Hexane:IPA	0.1 % DEA in MeOH:IPA	0.1 % DEA in CH_3CN:IPA
Acidic and Neutral Compounds	0.1 % HAC (or FA) in Hexane:IPA	0.1 % HAC (or FA) in MeOH:IPA	0.1 % HAC (or FA) in CH_3CN:IPA

IPA: Iso-propanol; **DEA:** Diethylamine; **HAC:** Acetic Acid; **FA:** Formic acid; **MeOH:** Methanol; **CH_3CN:** Acetonitrile

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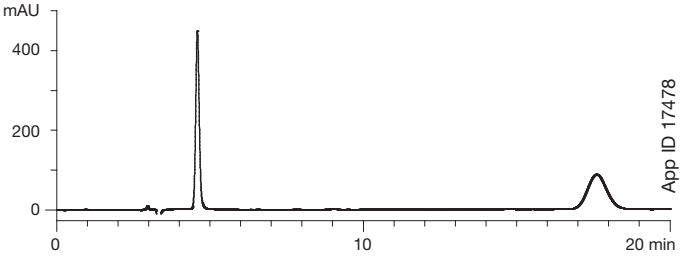
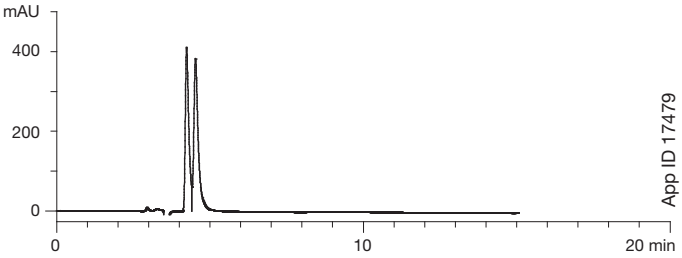
Figure 2.
Enantioseparations of ß-Blockers in Normal Phase

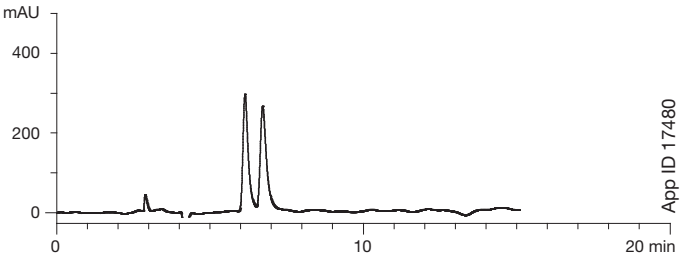
Toliprolol on Lux Cellulose-1
0.1 % DEA in Hexane / 0.1 % DEA in IPA (80:20)



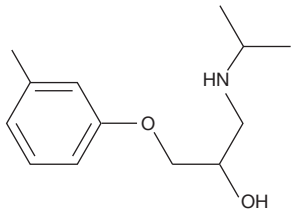
Toliprolol on Lux Cellulose-2
0.1 % DEA in Hexane / 0.1 % DEA in IPA (80:20)



Toliprolol on Lux Cellulose-2
0.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)

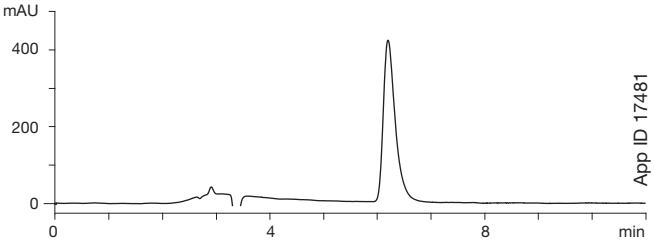


Column: Lux 5 µm Cellulose-1
Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

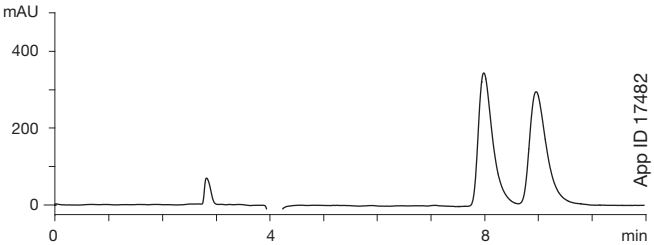


Toliprolol

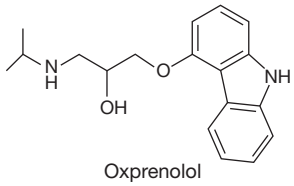
Oxprenolol on Lux Cellulose-1
0.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)



Oxprenolol on Lux Cellulose-2
0.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)

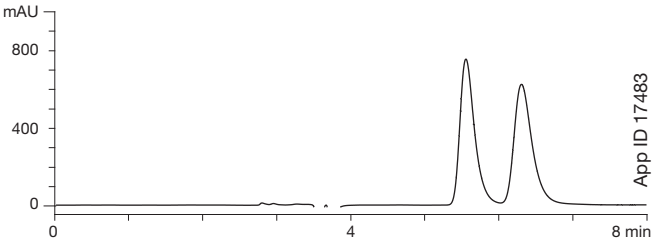


Column: Lux 5 µm Cellulose-1
Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

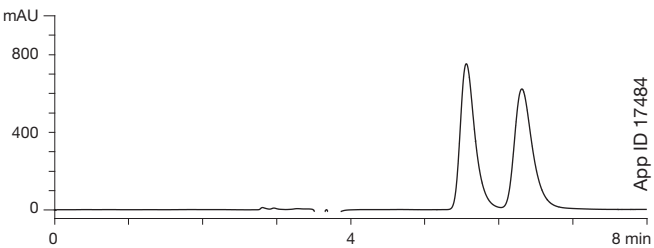


Oxprenolol

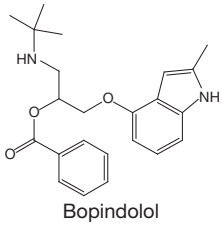
Bopindolol on Lux Cellulose-1
0.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)



Bopindolol on Lux Cellulose-2
0.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)



Column: Lux 5 µm Cellulose-1
Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



Bopindolol

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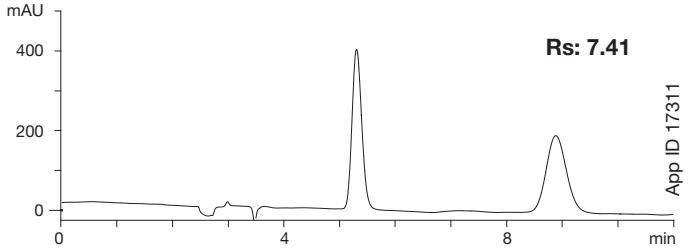
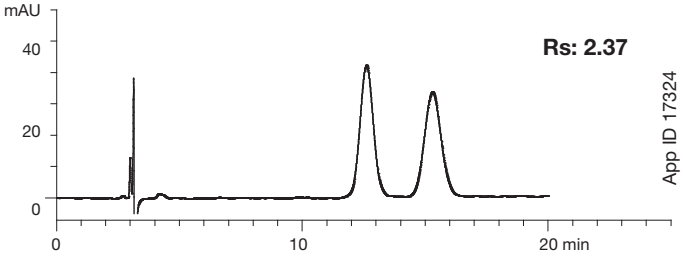
Figure 3.
Enantioseparations in Normal Phase

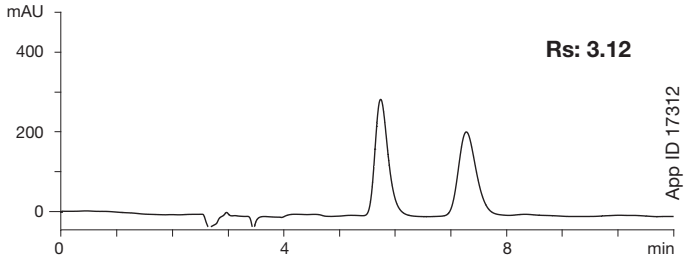
Warfarin on Lux Cellulose-1
0.1 % Formic Acid in Hexane / 0.1 % Formic Acid in IPA (60:40)



Sulcanozole on Lux Cellulose-1
0.1 % DEA in Hexane / 0.1 % DEA in IPA (60:40)

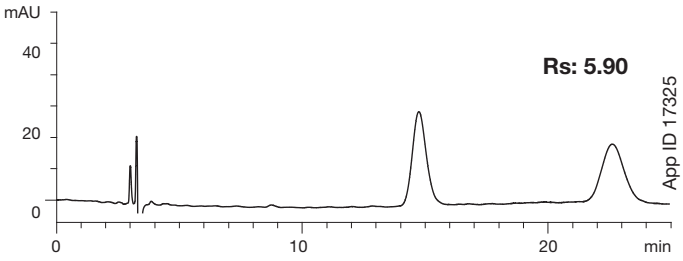


Warfarin on Lux Cellulose-2
0.1 % Formic Acid in Hexane / 0.1 % Formic Acid in IPA (60:40)



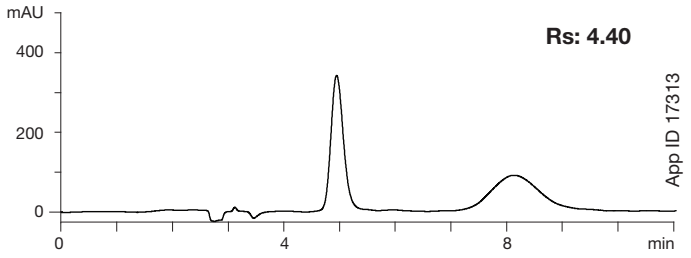
Column: Lux 5 µm Cellulose-1
Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

Sulcanozole on Lux Cellulose-2
0.1 % DEA in Hexane / 0.1 % DEA in IPA (60:40)

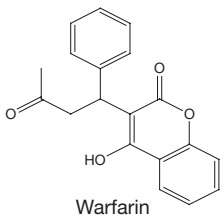


Column: Lux 5 µm Cellulose-1
Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

Warfarin on CHIRALCEL®† OD-H®
0.1 % Formic Acid in Hexane / 0.1 % Formic Acid in IPA (60:40)

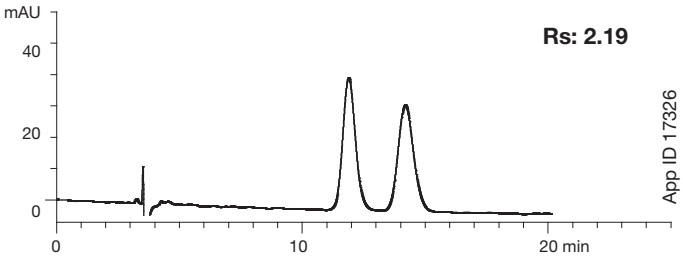


Column: CHIRALCEL® 5 µm OD-H®
Dimensions: 250 x 4.6 mm ID
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

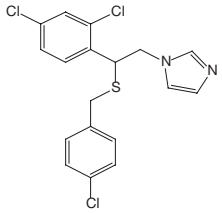


Warfarin

Sulcanozole on CHIRALCEL®† OD-H®
0.1 % DEA in Hexane / 0.1 % DEA in IPA (60:40)



Column: CHIRALCEL® 5 µm OD-H®
Dimensions: 250 x 4.6 mm ID
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



Sulconazole

*with 0.1 % Formic Acid
†CHIRALCEL and OD-H are registered trademarks of DAICEL Chemical Industries, Ltd. Comparative separations may not be representative of all applications.

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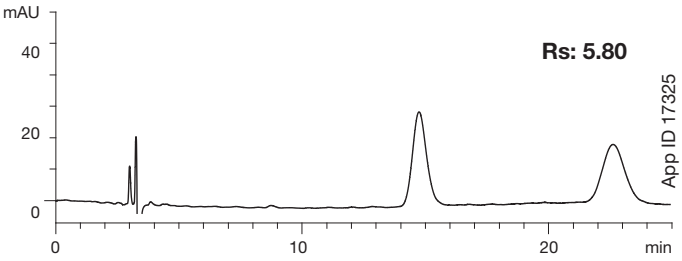
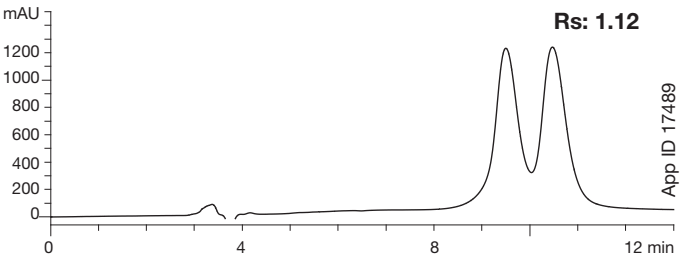
Figure 4.
Complementary Enantioselectivity in Normal Phase and Polar-Organic

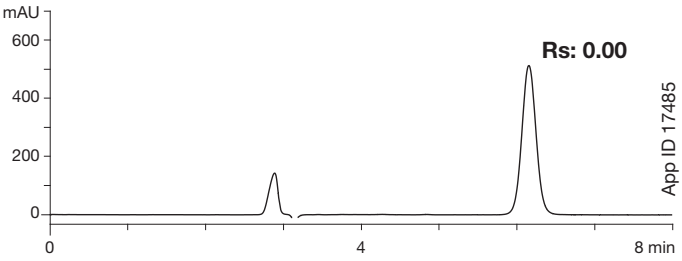
Sulcanozole on Lux Cellulose-2
0.1 % DEA in Hexane / 0.1 % DEA in IPA (60:40)



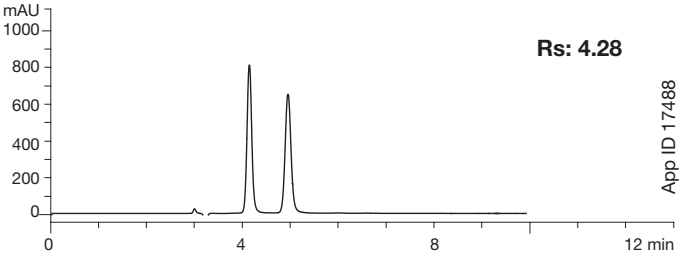
Milnacipran on Lux Cellulose-2
0.1 % DEA in Hexane / 0.1 % DEA in IPA (80:20)



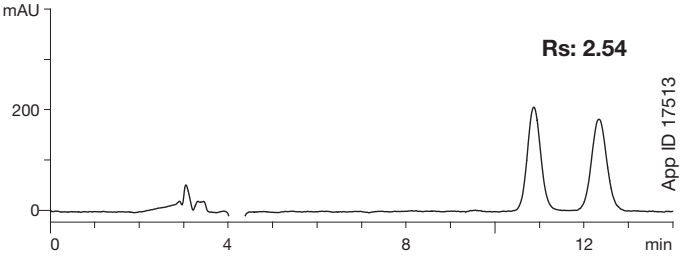
Sulcanozole on Lux Cellulose-2
0.1 % DEA in MeOH / 0.1 % DEA in IPA (95:5)



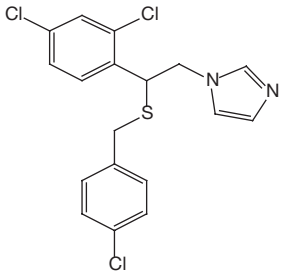
Milnacipran on Lux Cellulose-2
0.1 % DEA in MeOH / 0.1 % DEA in IPA (90:10)



Sulcanozole on Lux Cellulose-2
0.1 % DEA in CH_3CN / 0.1 % DEA in IPA (95:5)

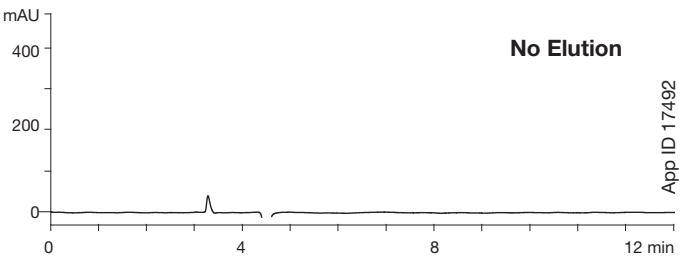


Column: Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

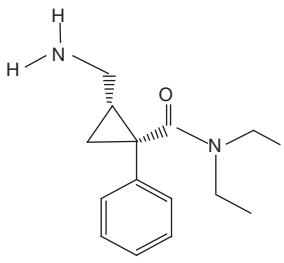


Sulconazole

Milnacipran on Lux Cellulose-2
0.1 % DEA in CH_3CN / 0.1 % DEA in IPA (95:5)



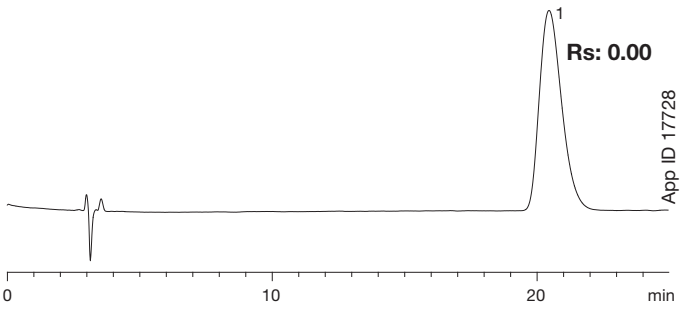
Column: Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



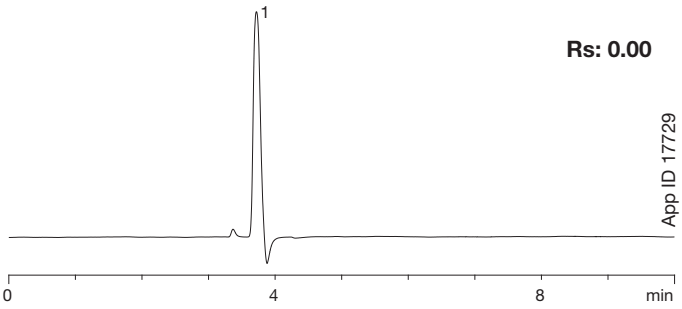
Milnacipran

Figure 4. (cont'd)
Complementary Enantioselectivity in Normal Phase and Polar-Organic

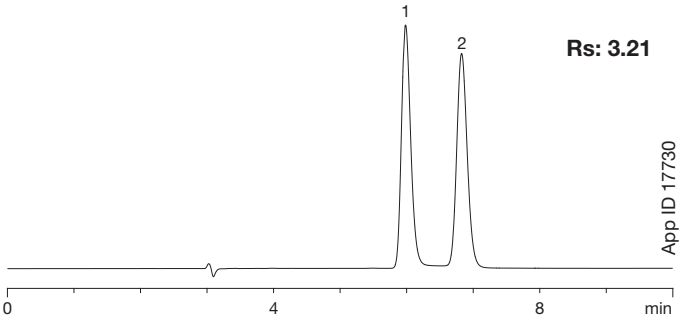
Chlormezanone on Lux Cellulose-1
0.1 % DEA in CH3CN / 0.1 % DEA in IPA (60:40)



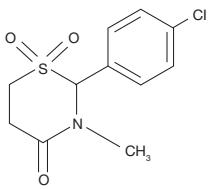
Chlormezanone on Lux Cellulose-1
0.1 % DEA in CH3CN / 0.1 % DEA in IPA (95:5)



Chlormezanone on Lux Cellulose-1
0.1 % DEA in MeOH / 0.1 % DEA in IPA (90:10)



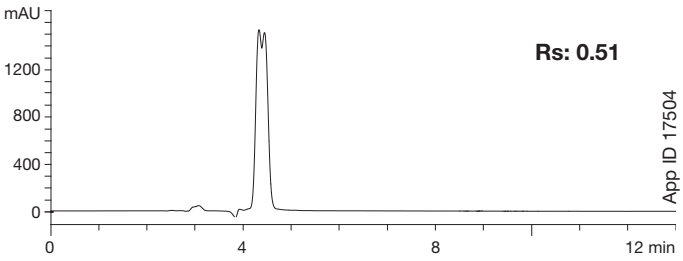
Column: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



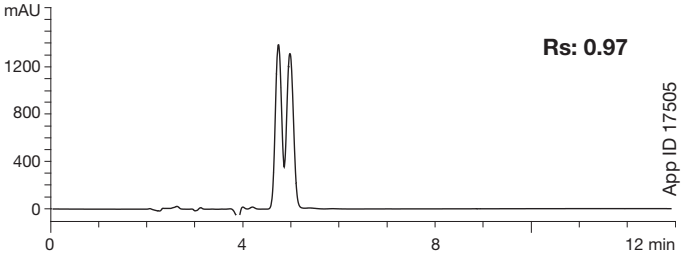
Chlormezanone

Figure 5.
Enantioseparations in Reversed Phase

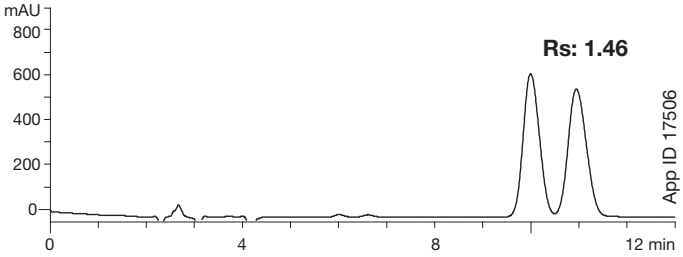
Clenbuterol on Lux Cellulose-2
0.1 % DEA in MeOH / 0.1 % DEA in Water (80:20)



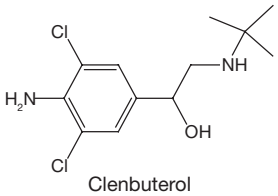
Clenbuterol on Lux Cellulose-2
0.1 % DEA in CH_3CN / 0.1 % DEA in Water (60:40)



Clenbuterol on Lux Cellulose-2
0.1 % DEA in CH_3CN / 0.1 % DEA in Water (40:60)



Column: Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



Clenbuterol

Mobile Phase	Rs
Hexane:IPA	0.00
MeOH:IPA	0.00
CH_3CN	0.00

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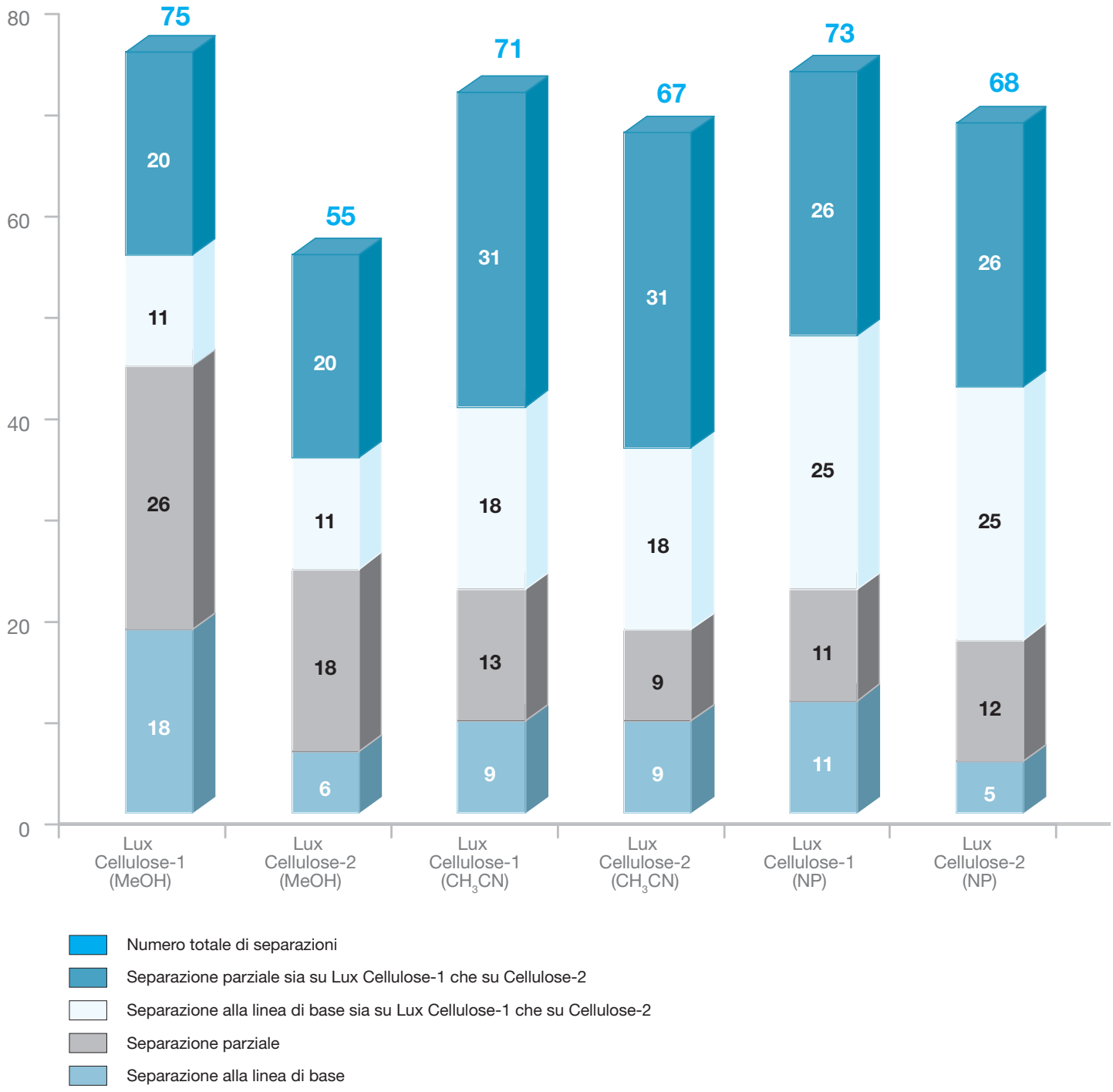
Figure 6.
Success Rates for over 180 Racemates on Lux™ Cellulose-1 and -2



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Results and Discussion

Lux, a new line of polysaccharide-based chiral selective phases, has recently been introduced into the market. One phase, Lux™ Cellulose-1 is based on cellulose tris (3,5- dimethylphenyl carbamate) similar to other chiral phases on the market (e.g. CHIRACEL® OD-H®). The other phase, Lux Cellulose-2, is a new member to the family of polysaccharide based chiral selective phases and uses cellulose tris (3-chloro-4-methylphenyl carbamate) (**Figure 1**) as a chiral selector; this new chemistry delivers a unique selectivity versus other phases 2,3.

In this study over 180 diverse compounds of pharmaceutical interest were screened on the Lux line of chiral selective phases as well as other comparative medias to better characterize the selectivity delivered by each Lux phase.

Table 1 summarizes the screening conditions used for each column; different types of mobile phases (NP, PO, and RP) as well as additives used (0.1 formic acid or acetic acid for acidic analytes or 0.1% diethylamine for basic analytes) .

Figures 2-5 show several representative examples of the different selectivities provided by Lux Cellulose-1 and Lux Cellulose-2 in chiral separations across normal phase, polar-organic and reversed phase separation modes. Representative compounds such as various ß-blockers, warfarin, sulconazole, milnacipran, and clenbuterol demonstrate the complementary behavior of Lux Cellulose-2 to the commonly used cellulose tris (3.5-dimethylphenylcarbamate) based CSPs (CHIRACEL® OD-H® and Lux Cellulose-1) in the separation of difficult racemates.

Figure 2 demonstrates the behavior of the two Lux phases in normal phase separations. While Lux Cellulose-1 generally demonstrates slightly better resolution and increased retention versus CHIRACEL® OD-H®, there are several cases where Lux Cellulose-2 is a better separation choice when using normal phase. While Bopindolol is equally well separated on the two Lux phases, Oxprenolol enantiomers are better resolved on Lux Cellulose-2. Toliprolol enantomers are separated on Lux Celluose-1 with spectacular resolution but at the expense of extensive retention for one of the enantomers. Lux Cellulose-2 barely separates racemic Oxprenolol under similar mobile phase conditions, but with minimal optimization (i.e. a reduction of IPA in the mobile phase) a better separation is achieved with Lux Cellulose-2 with significantly shorter analysis time. **Figure 3** shows additional normal phase enantomeric separations using Lux Cellulose-1, Lux Cellulose-2 and CHIRACEL® OD-H®. Such separations further demonstrate the complementary selectivity offered by Lux Cellulose-2 versus the cellulose tris (3,5-dimethylphenyl carbamate) phases Lux Cellulose-1 and CHIRACEL® OD-H®.

Figures 4 and **5** demonstrate the effect of mobile phase composition on chiral resolution. **Figure 4** demonstrates the dramatic changes in selectivity for each phase when traditional normal phase separation is substituted with polar organic separation mode using either acetonitrile or methanol as mobile phase. **Figure 5** shows changes in selectivity are observed in reversed phase mode. Different solvents can alter the steric structure of the polysaccharide backbone and the arrangement of binding sites[4], providing alternative selectivity for separating difficult chiral compounds. Such mobile phase alteration offers a powerful tool in developing and optimizing chiral separations.

Figure 6 compares the success rates of Lux Cellulose-1 and Lux Cellulose-2 in the analysis of over 180 racemates in normal phase or polar organic separation modes. The number of uniquely baseline resolved racemates are given at the bottom of the bar graph. The same selection criteria was applied to partially separated racemates. For example, Lux Cellulose-2 shows good chiral recognition in acetonitrile mobile phase with 9 baseline separations of racemates that could not be separated on Lux Cellulose-1. This complementary enantioselectivity of Lux Cellulose-2 over Lux Cellulose-1 is most evident in acetonitrile: IPA mobile phase mixtures, and is less pronounced in standard normal phase mixtures (hexane / IPA) and methanol mixtures.

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Ordering Information

Lux 5 µm Analytical Columns (mm)

		100 x 4.6	150 x 4.6	250 x 4.6
	Phase			
	Cellulose-1	00D-4459-E0	00F-4459-E0	00G-4459-E0
	Cellulose-2	00D-4457-E0	00F-4457-E0	00G-4457-E0
NEW	**Amylose-2**	00D-4472-E0	00F-4472-E0	00G-4472-E0

Lux phases are available in 3 µm, 5 µm and 20 µm and many other dimensions.

APPLICATIONS

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium
t: +31 (0)30-2418700
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: (800) 543-3681
f: (310) 328-7768
info@phenomenex.com

Denmark
t: 4824 8048
f: 4810 6265
dkinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Netherlands
t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

Puerto Rico
t: (800) 541-HPLC
f: (310) 328-7768
info@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

All other countries:
Corporate Office USA
t: (310) 212-0555
f: (310) 328-7768
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