## TN-1047

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

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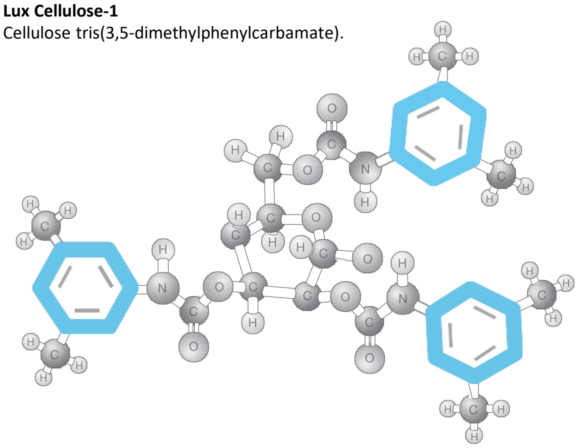
**Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA**

## Introduction

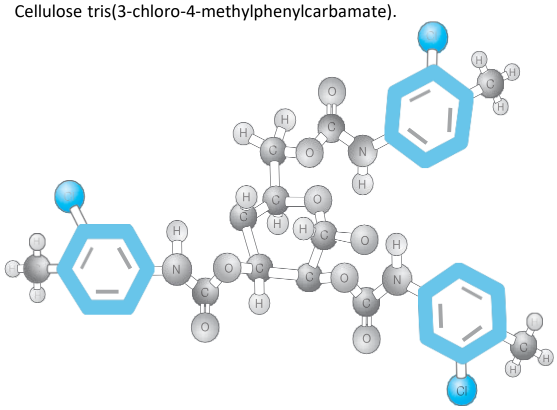
Polysaccharide-based chiral stationary phases (CSP) are widely used due to their wide chiral recognition ability. Several cellulose and amylose derivatives are extremely effective in the separation of a wide range of compounds of interest in the pharmaceutical industry. This technical note demonstrates 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 phase (NP), polar-organic (PO) and reversed phase (RP) separation modes. Numerous examples including important classes of drug compounds as well as statistical data demonstrates 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-chloro-4-methylphenylcarbamate).



## LC Conditions

**Column:** Lux™ 5 µm Cellulose-1 (00G-4459-E0)
Lux 5 µm Cellulose-2 (00G-4457-E0)
CHIRALCEL® 5 µm OD-H®
**Dimensions:** 250 x 4.6 mm
**Mobile Phase:** See **Table 1**
**Flow Rate:** 1.0 mL/min (Isocratic)
**Injection Volume:** 5 - 20 µL (depending on analyte response)
**Temperature:** Ambient
**LC System:** Agilent® 1100
**Detection:** UV @ 220 nm
**Sample:** 500 µg/mL racemate dissolved in mobile phase

**Table 1.** Mobile Phase Compositions.

| Mobile Phase | Basic and Neutral Compounds | Acidic and Neutral Compounds |
|---|---|---|
| NP | 0.1 % Diethylamine in Hexane / Isopropanol | 0.1 % Acetic Acid (or Formic Acid) in Hexane / Isopropanol |
| PO | 0.1 % Diethylamine in Methanol / Isopropanol | 0.1 % Acetic Acid (or Formic Acid) in Methanol / Isopropanol |
| | 0.1 % Diethylamine in Acetonitrile / Isopropanol | 0.1 % Acetic Acid (or Formic Acid) in Acetonitrile / Isopropanol |
| RP | 0.1 % Diethylamine in Acetonitrile / Water | 0.1 % Acetic Acid (or Formic Acid) in Acetonitrile / Water |
| | 0.1 % Diethylamine in Methanol / Water | 0.1 % Acetic Acid (or Formic Acid) in Methanol / Water |

## Results and Discussion

Lux™, the Phenomenex brand of polysaccharide-based chiral selective phases, was introduced into the market in 2008. One phase, Lux Cellulose-1 is based on Cellulose tris(3,5-dimethylphenyl carbamate) like other chiral phases on the market (e.g., CHIRALCEL® OD-H®). Another 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.

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 or PO) 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, Sulcanozole, Milnacipran, and Clenbuterol demonstrate the complementary behavior of Lux Cellulose-2 to the commonly used cellulose tris(3.5-dimethylphenylcarbamate) based CSPs (CHIRALCEL OD-H and Lux Cellulose-1) in the separation of difficult racemates.

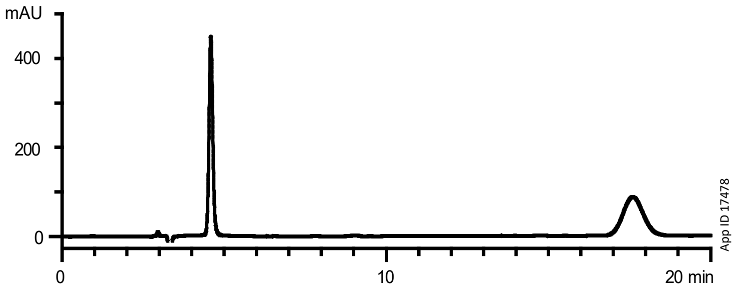
**Figure 2** demonstrates the behavior of the two Lux phases in normal phase separations. While Lux Cellulose-1 generally demonstrates slightly better resolution and increased retention versus CHIRALCEL OD-H, there are several cases where Lux Cellulose-2 is a better separation choice when using normal phase. While Bopindolol is equally well separated on the two Lux phases, Oxprenolol enantiomers are better resolved on Lux Cellulose-2. Toliprolol enantiomers are separated on Lux Celluose-1 with spectacular resolution but at the expense of extensive retention for one of the enantiomers. Lux Cellulose-2 barely separates racemic Oxprenolol under similar mobile phase conditions, but with minimal optimization (i.e. a reduction of Isopropanol in the mobile phase) a better separation is achieved with Lux Cellulose-2 with significantly shorter analysis time. **Figure 3** shows additional normal phase enantiomeric separations using Lux Cellulose-1, Lux Cellulose-2, and CHIRALCEL 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 CHIRALCEL 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, providing alternative selectivity for separating difficult chiral compounds. Such mobile phase alteration offers a powerful tool in developing and optimizing chiral separations.

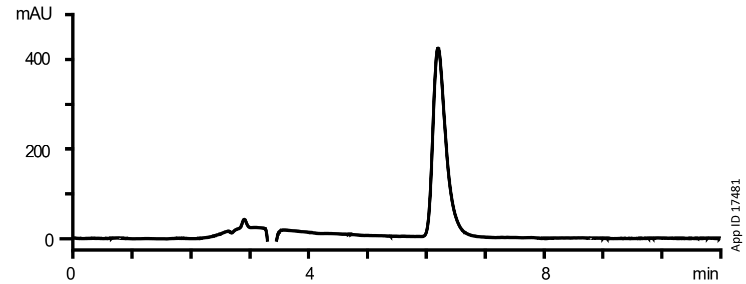
**Figure 6** compares the success rates of Lux Cellulose-1 and Lux Cellulose-2 in the analysis of over 180 racemates in normal phase or polar organic separation modes. The number of uniquely baseline resolved racemates are given at the bottom of the bar graph. The same selection criteria was applied 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 / Isopropanol mobile phase mixtures and is less pronounced in standard normal phase mixtures (Hexane / Isopropanol) and Methanol mixtures.

**Figure 2.** Enantioseparations of β-Blockers in Normal Phase.

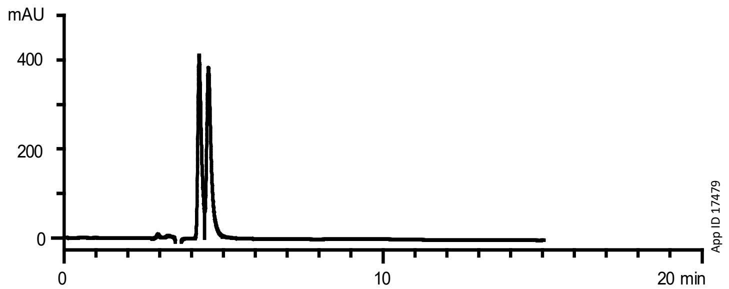
Toliprolol on Lux™ Cellulose-1
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (80:20, v/v)



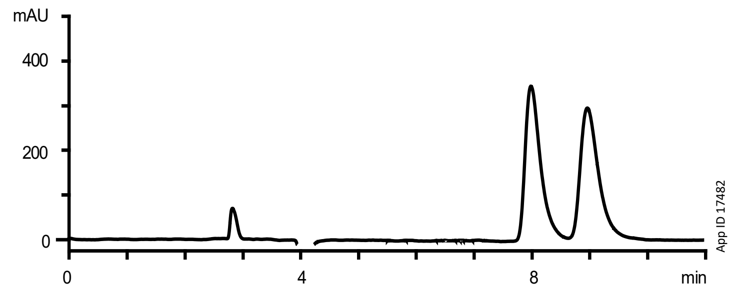

Oxprenolol on Lux Cellulose-1
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (90:10, v/v)



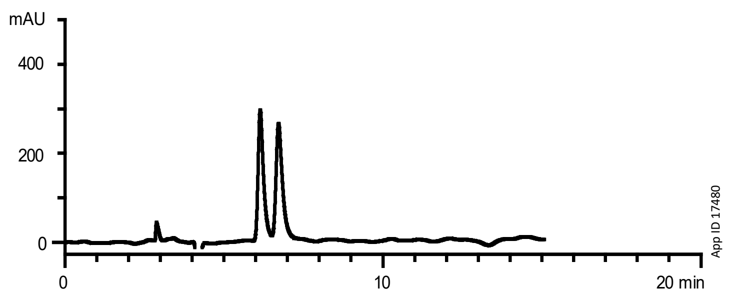

Toliprolol on Lux Cellulose-2
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (80:20, v/v)



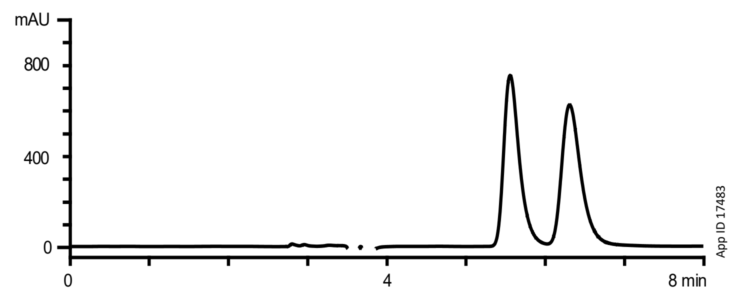

Oxprenolol on Lux Cellulose-2
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (90:10, v/v)



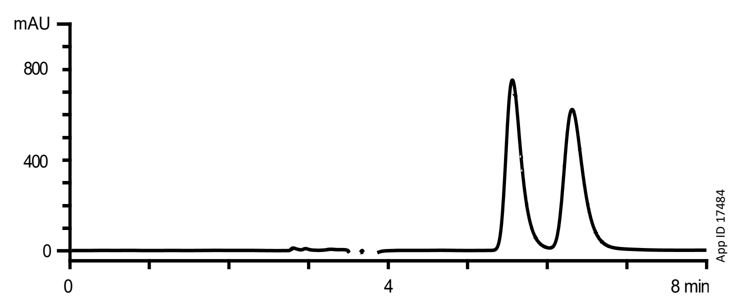

Toliprolol on Lux Cellulose-2
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (90:10, v/v)

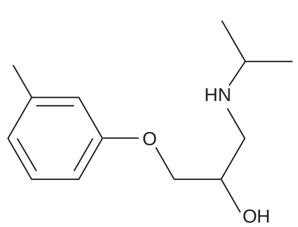



Bopindolol on Lux Cellulose-1
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (90:10, v/v)

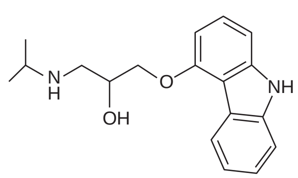



Bopindolol on Lux Cellulose-2
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (90:10, v/v)

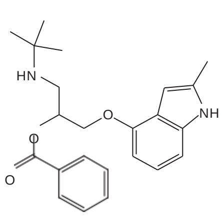



Toliprolol




Oxprenolol




Bopindolol

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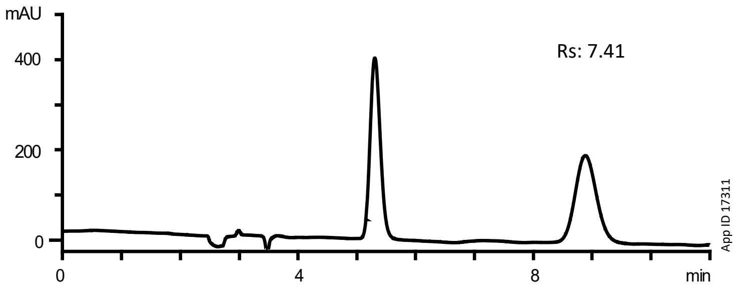
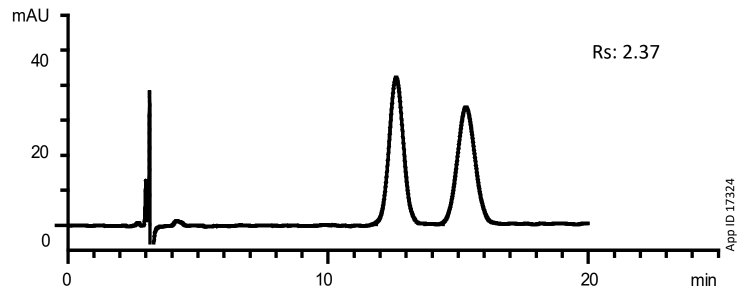
**Figure 3 .** Enantioseparations in Normal Phase.

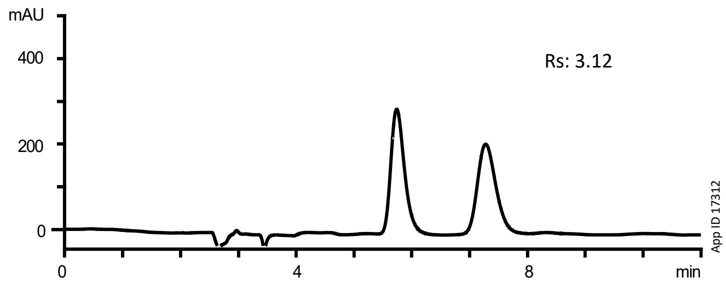
Warfarin on Lux™ Cellulose-1
0.1 % Formic Acid in Hexane / 0.1 % Formic Acid in Isopropanol (60:40, v/v)



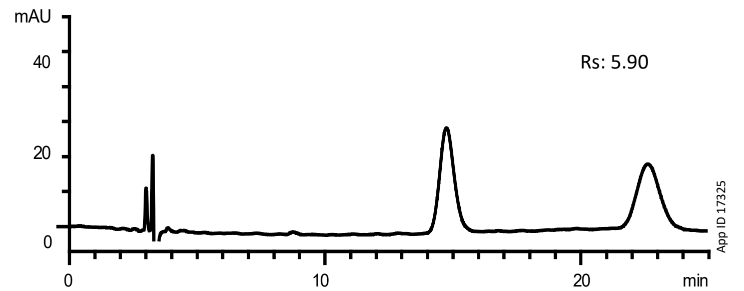

Sulcanozole on Lux Cellulose-1
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (60:40, v/v)



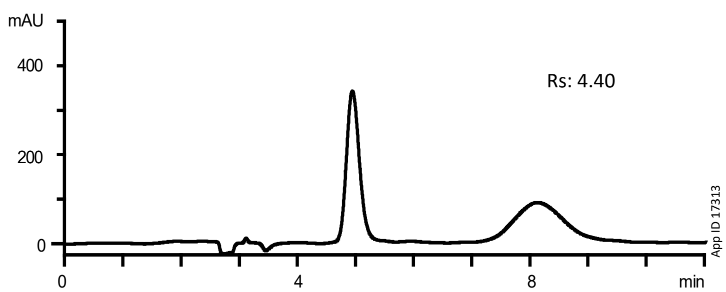

Warfarin on Lux Cellulose-2
0.1 % Formic Acid in Hexane / 0.1 % Formic Acid in Isopropanol (60:40, v/v)



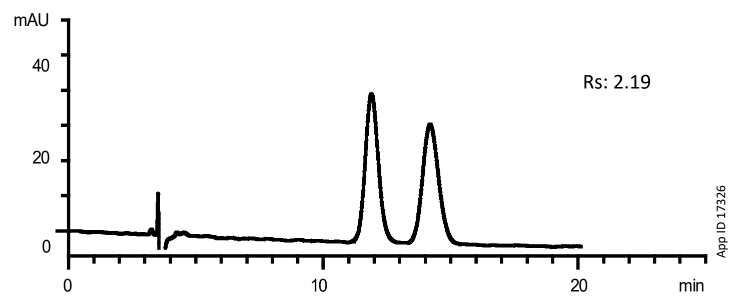

Sulcanozole on Lux Cellulose-2
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (60:40, v/v)

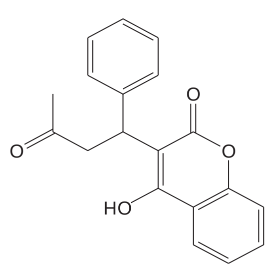



Warfarin on CHIRALCEL® OD-H®
0.1 % Formic Acid in Hexane / 0.1 % Formic Acid in Isopropanol (60:40, v/v)

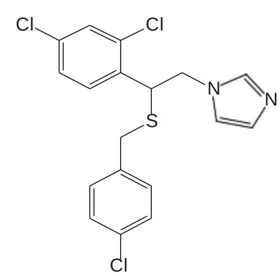



Sulcanozole on CHIRALCEL OD-H
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (60:40, v/v)



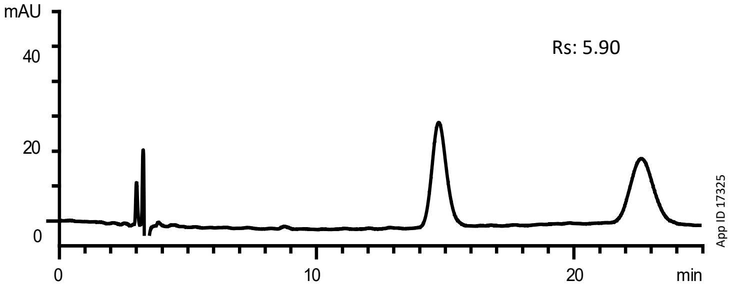

Warfarin



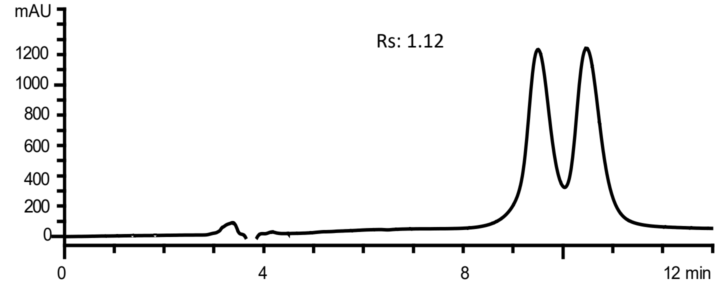

Sulcanozole

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

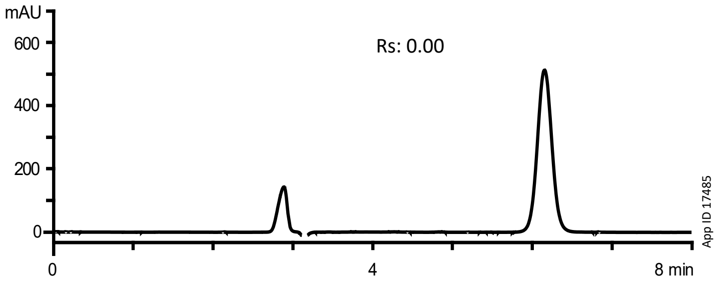
Sulcanozole on Lux™ Cellulose-2
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (60:40, v/v)



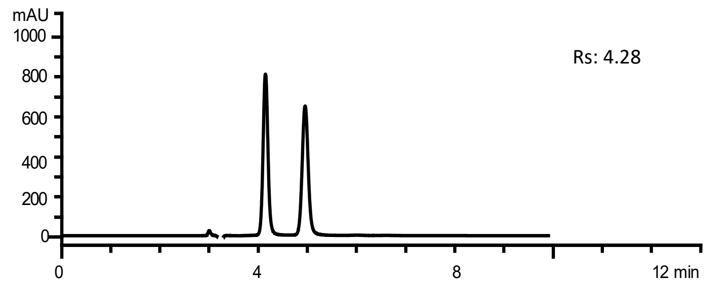

Milnacipran on Lux Cellulose-2
0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Isopropanol (80:20, v/v)



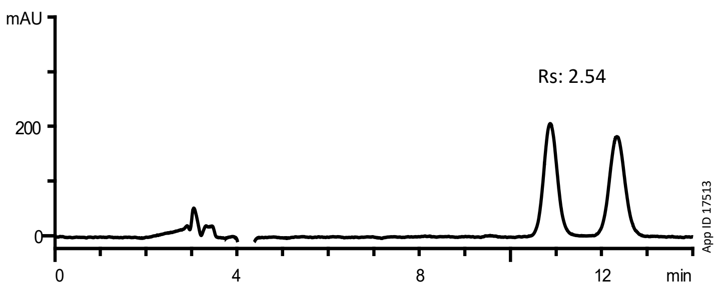

Sulcanozole on Lux Cellulose-2
0.1 % Diethylamine in Methanol / 0.1 % Diethylamine in Isopropanol (95:5, v/v)



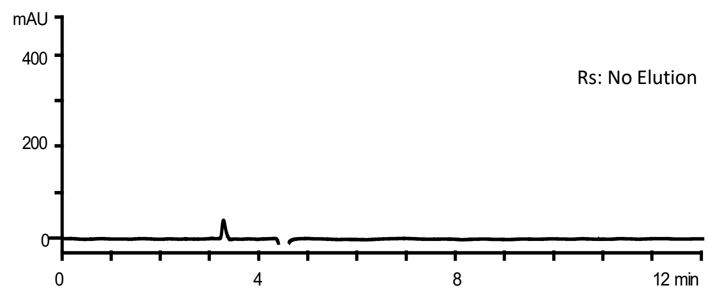

Milnacipran on Lux Cellulose-2
0.1 % Diethylamine in Methanol / 0.1 % Diethylamine in Isopropanol (90:10, v/v)

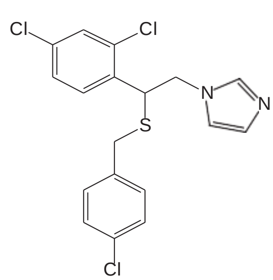



Sulcanozole on Lux Cellulose-2
0.1 % Diethylamine in Acetonitrile / 0.1 % Diethylamine in Isopropanol (95:5, v/v)

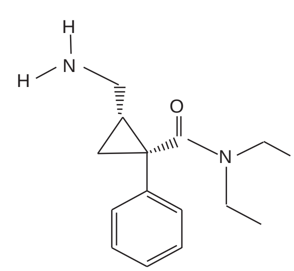



Milnacipran on Lux Cellulose-2
0.1 % Diethylamine in Acetonitrile / 0.1 % Diethylamine in Isopropanol (95:5, v/v)




Sulcanozole




Milnacipran

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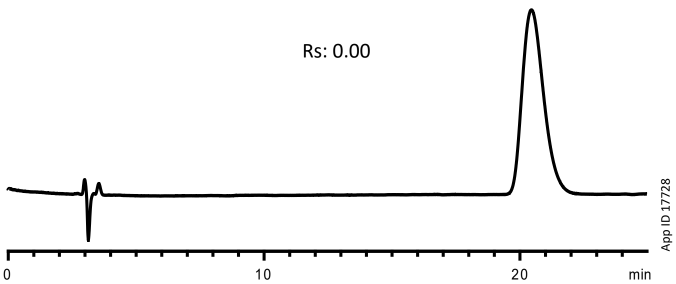
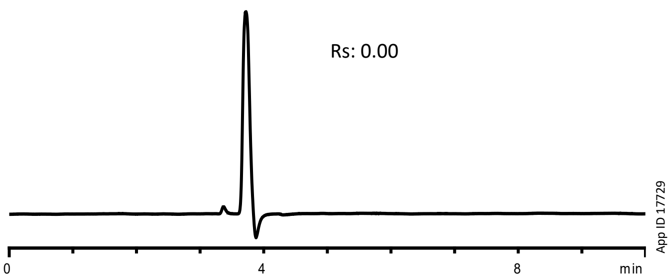
**Figure 4 Cont'd.** Complementary Enantioselectivity in Normal Phase and Polar-Organic.

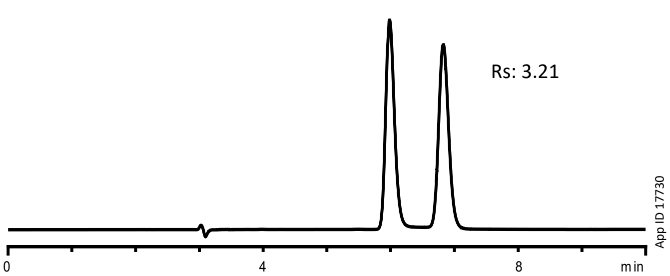
Chlormezanone on Lux™ Cellulose-1
0.1 % Diethylamine in Acetonitrile / 0.1 % Diethylamine in Isopropanol (60:40, v/v)

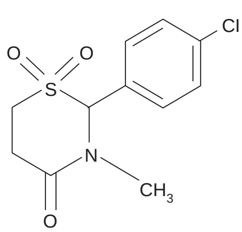



Chlormezanone on Lux Cellulose-2
0.1 % Diethylamine in Acetonitrile / 0.1 % Diethylamine in Isopropanol (95:5, v/v)




Chlormezanone on Lux Cellulose-1
0.1 % DIethylamine in Methanol / 0.1 % Diethylamine in Isopropanol (90:10, v/v)

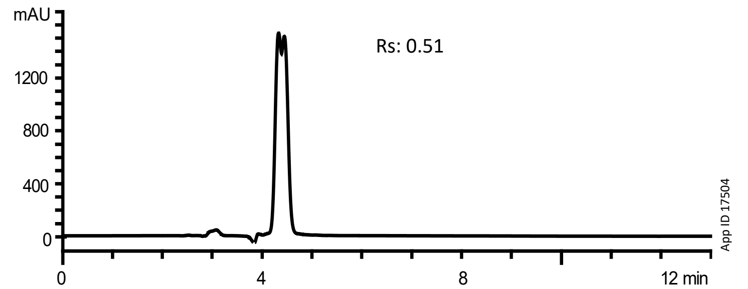


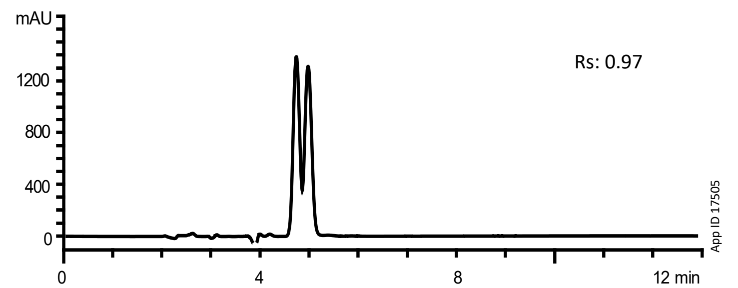

Chlormezanone

**Figure 5.** Enantioseparations in Reversed Phase.

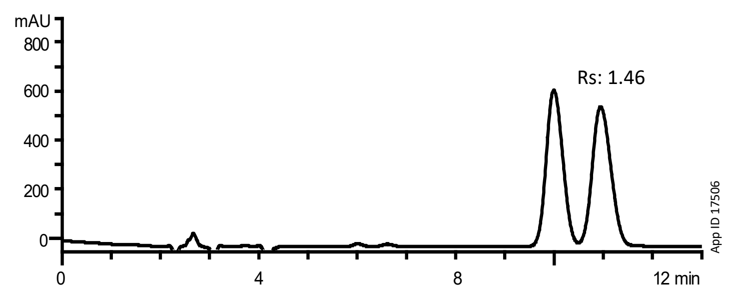
Clenbuterol on Lux Cellulose-2
0.1 % Diethylamine in Methanol / 0.1 % Diethylamine in Water (80:20, v/v)

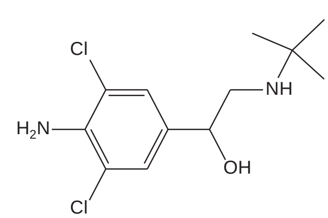



Clenbuterol on Lux Cellulose-2
0.1 % Diethylamine in Acetonitrile / 0.1 % Diethylamine in Water (60:40, v/v)




Clenbuterol on Lux Cellulose-2
0.1 % Diethylamine in Acetonitrile / 0.1 % Diethylamine in Water (40:60, v/v)

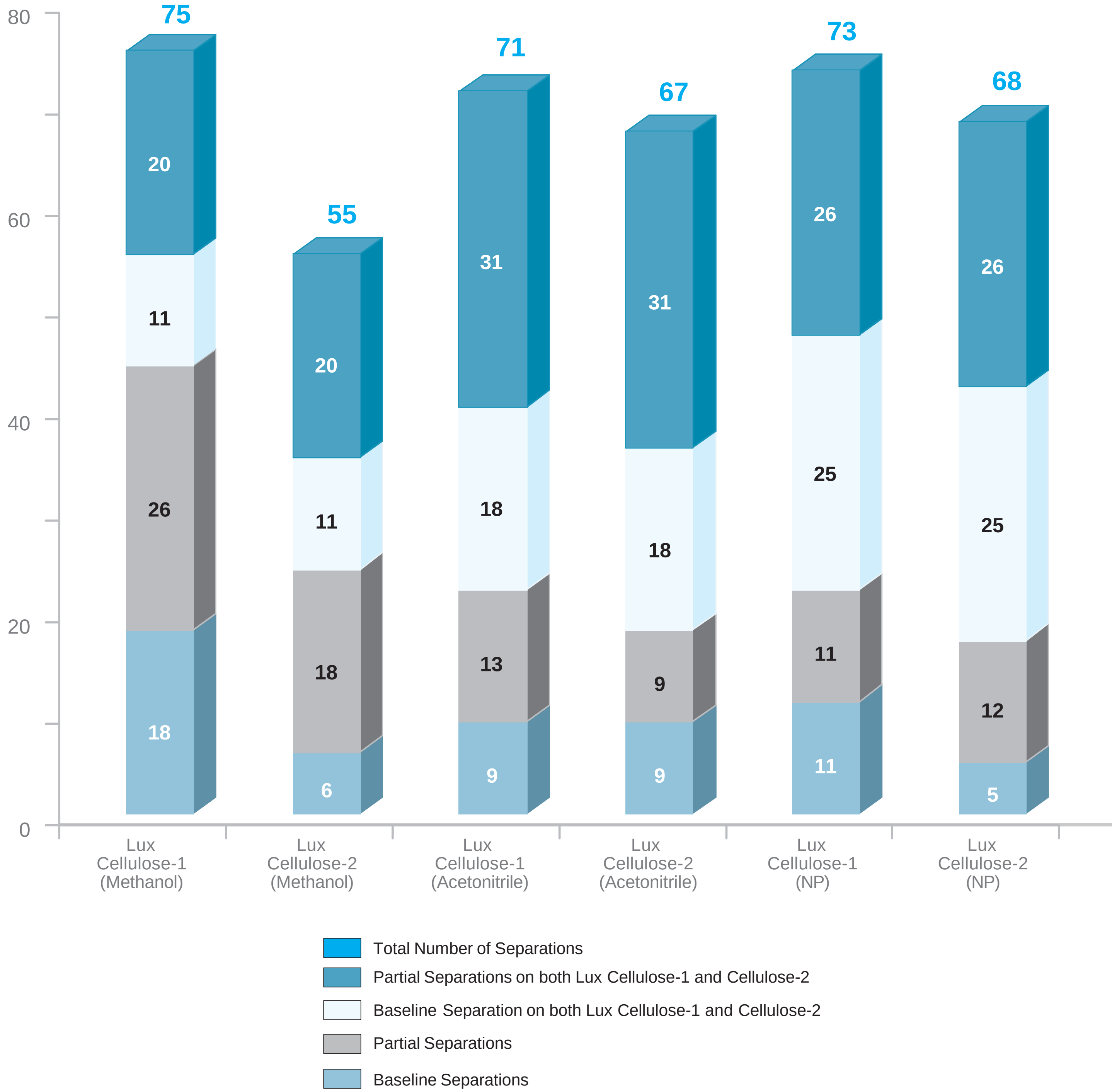



Clenbuterol

| Mobile Phase | Rs |
|---|---|
| Hexane / Isopropanol | 0.00 |
| Methanol / Isopropanol | 0.00 |
| Acetonitrile | 0.00 |

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



## Lux™ Ordering Information

| 5 µm Minibore and 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* |
| | | | | | | /10pk | /10pk |
| i-Amylose-1 | 00B-4762-B0 | 00B-4762-E0 | 00D-4762-E0 | 00F-4762-E0 | 00G-4762-E0 | AJ0-8640 | AJ0-8641 |
| i-Amylose-3 | — | 00B-4779-E0 | 00D-4779-E0 | 00F-4779-E0 | 00G-4779-E0 | AJ0-8651 | AJ0-8650 |
| i-Cellulose-5 | — | 00B-4756-E0 | 00D-4756-E0 | 00F-4756-E0 | 00G-4756-E0 | AJ0-8631 | AJ0-8632 |
| Cellulose-1 | — | 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-E0 | 00D-4493-E0 | 00F-4493-E0 | 00G-4493-E0 | AJ0-8621 | AJ0-8622 |
| Cellulose-4 | — | — | 00D-4491-E0 | 00F-4491-E0 | 00G-4491-E0 | AJ0-8626 | AJ0-8627 |
| Amylose-1 | 00B-4732-B0 | — | 00D-4732-E0 | 00F-4732-E0 | 00G-4732-E0 | AJ0-9337 | AJ0-9336 |
| | | | | | for ID: | 2.0-3.0 mm | 3.2-8.0 mm |

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282



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### Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.


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t: +32 (0)2 511 8666 (Dutch)
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