# Chromatographic Enantioseparation of Racemic Pain Relievers using Lux® Polysaccharide-Based Chiral Stationary Phases

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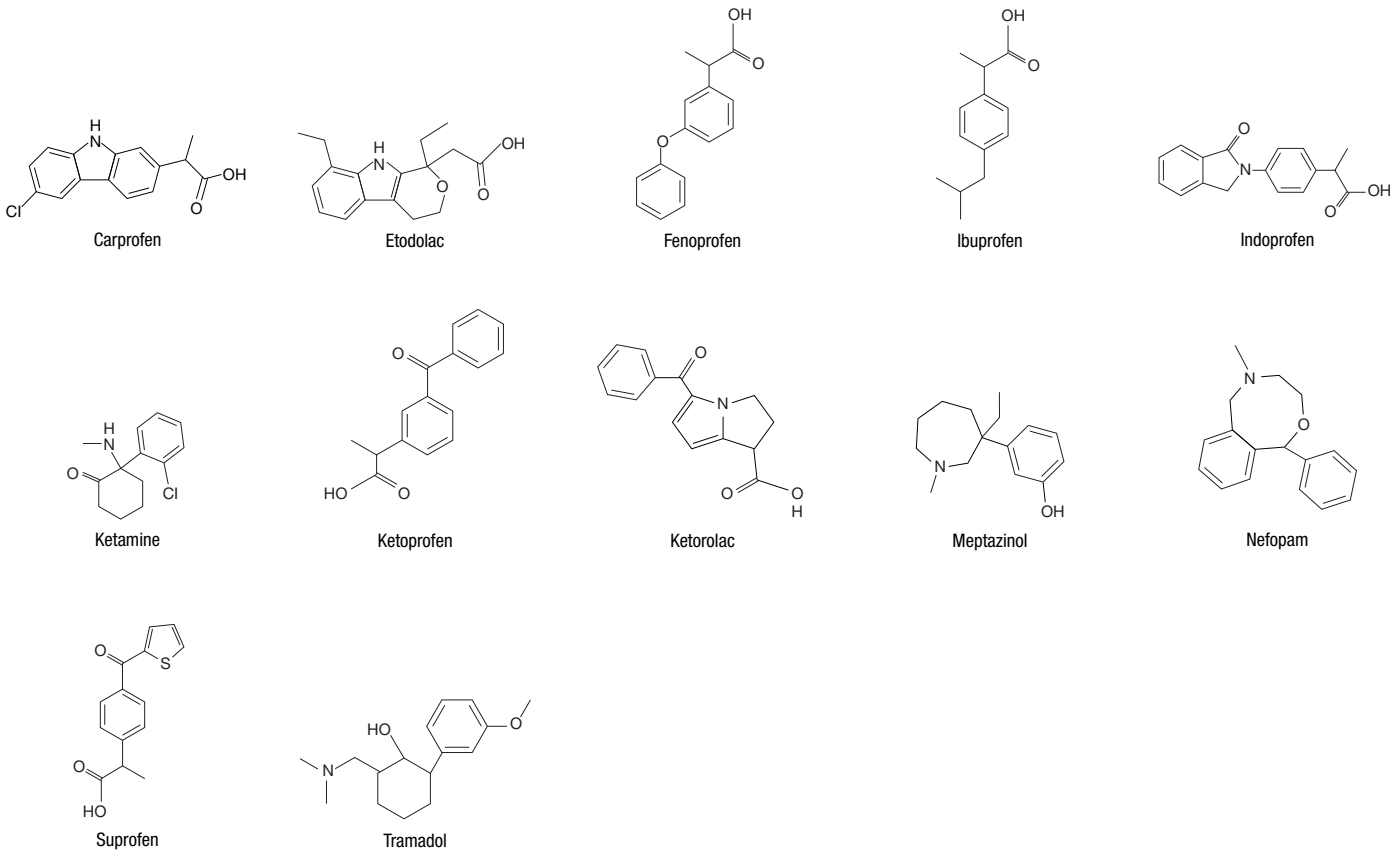
*In this technical note, we report the chiral chromatographic separation of various anti-inflammatory agents and pain relievers using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in polar organic, normal phase, and reversed phase separation modes.*

## Introduction

Chiral separations can be performed by chromatographic separation, enzymatic resolution, and crystallization. Chromatographic enantioselective separation using chiral stationary phases (CSPs) for high performance liquid chromatography (HPLC) has significantly evolved during the past few decades and is recognized as the most popular and reliable tool for both analytical and preparative separation of chiral compounds. Polysaccharide-based CSPs such as Lux are the most widely use CSPs for the chromatographic separation of enantiomers.[1] A recent review pointed out that in 2007 more than 90 % of the HPLC methods used for the determination of enantiomeric excess were performed on polysaccharide-based chiral stationary phases.[2] The polysaccharide-based CSPs are frequently used for preparative purifications because they are easily scaled-up from the analytical separations.[3]

Anti-inflammatory and analgesic agents are effective in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation. The pain relievers analyzed in this study are depicted in **Figure 1.** The chiral separations described in this application are the results of a systematic screening of our five Lux polysaccharide-based CSPs (Cellulose-1, Cellulose-2, Cellulose-3, Cellulose-4, and Amylose-2) under various separation modes.

**Figure 1.** Chemical structures of pain relievers and anti-inflammatory agents



# APPLICATIONS

## Material and Methods

All analyses were performed using an Agilent® 1100 series LC system (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with quaternary pump, in-line degasser, multi-wavelength UV detector and autosampler. Lux® columns used for analysis were obtained from Phenomenex (Torrance, CA, USA). The HPLC column dimensions were 250 x 4.6 mm ID and all columns were packed with 5 µm particles. The flow rate was 1.0 mL/min and temperature was ambient. Standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were purchased from EMD (San Diego, CA, USA).

## Results and Discussion

Pain reliever agents depicted in **Figure 1** were analyzed on five different Lux polysaccharide-based CSPs (Cellulose-1, Cellulose-2, Cellulose-3, Cellulose-4, and Amylose-2) in normal phase (NP), polar organic (PO), and reversed phase (RP) separation modes. After performing a systematic screening with various mobile phases, the best separation was selected, even though in most of the cases alternative separation was obtained with other Lux phases and/or modes.

The racemic pain relievers analyzed in this study are listed in **Table 1**. For each compound tested we provide the chemical identification number (CID) of the racemate. This unique number can be linked to The PubChem Project website for further research regarding each compound's pharmaceutical properties. The table summarizes the Lux phases used, the selectivity, the retention time of the first enantiomer, as well as the isocratic conditions used for each compound. Lux columns are quite successful at resolving chiral drugs of this type. All the anti-inflammatory agents and pain relievers tested are separated with selectivity greater than 1.1. In the last column, the corresponding Phenomenex application number is provided. Those applications are easily accessible on our website (www.phenomenex.com/ChiralAppSearch) and can be searched by application number, structure, CID, or compound name.

The chiral separations reported in **Table 1** are baseline resolved with a resolution greater than 1.5. The retention time for the first enantiomer is between 5 and 14 min and all the separations are completed in less than 30 min. With basic pain relievers, 0.1% of diethylamine (DEA) was used as an additive, whereas with acidic derivatives, 0.1% of formic acid (FA) was used as additive. Interestingly with pain reliever drugs, Lux Cellulose-3 phase was quite successful. Out of the 12 separations, 9 were most successful with Lux Cellulose-3.

All of our Lux® products are pressure stable up to 300 bar and compatible with SFC separation mode[4] using an organic modifier such as MeOH, EtOH, IPA, or ACN. Two examples of chiral separation for Ibuprofen and Ketoprofen are shown in **Figure 2**.

**Table 1.** Chiral separations of anti-inflammatory agents using Lux polysaccharide-based CSPs

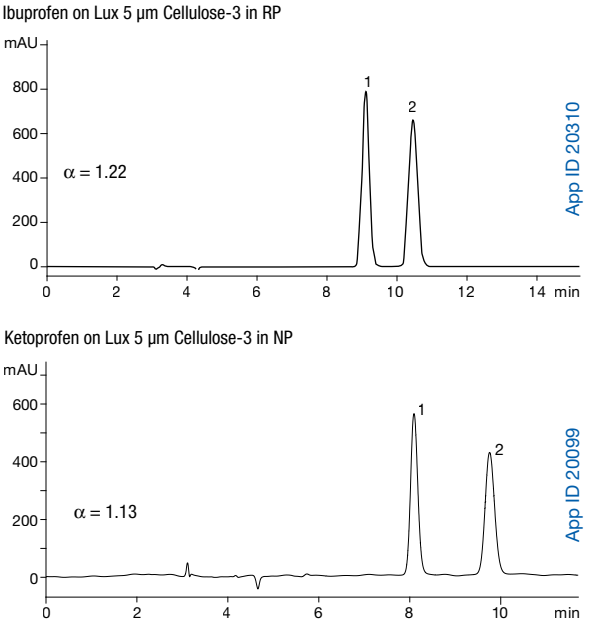
| Compound | CID | CSPs | (α) | Rt (min) | Mode | Mobile Phase | App ID* |
|---|---|---|---|---|---|---|---|
| Carprofen | 2581 | Lux Cellulose-3 | 1.20 | 7.69 min | NP | Hex/EtOH (60:40) FA (0.1%) | 20385 |
| Etodolac | 3308 | Lux Cellulose-3 | 1.21 | 7.38 min | RP | ACN/FA (0.1%) (40:60) | 20324 |
| Fenoprofen | 3342 | Lux Amylose-2 | 1.57 | 8.06 min | NP | Hexane/EtOH (95:5) FA (0.1%) | 20453 |
| Ibuprofen | 3672 | Lux Cellulose-3 | 1.22 | 9.1 min | RP | MeOH/FA (0.1%) (80:20) | 20310 |
| Indoprofen | 3718 | Lux Cellulose-3 | 1.17 | 12.29 min | RP | MeOH/FA (0.1%) (80:20) | 20296 |
| Ketamine | 3821 | Lux Cellulose-3 | 2.42 | 5.49 min | RP | MeOH/20 mM $NH_4HCO_3$ (90:10) DEA (0.1%) | 20287 |
| Ketoprofen | 3825 | Lux Cellulose-3 | 1.13 | 8.09 min | NP | Hex/IPA (80:20) FA (0.1%) | 20099 |
| Ketorolac | 3826 | Lux Cellulose-3 | 1.52 | 8.65 min | PO | MeOH/IPA (90:10) FA (0.1%) | 20367 |
| Meptazinol | 41049 | Lux Cellulose-3 | 1.38 | 7.74 min | NP | Hex/IPA (90:10) DEA (0.1%) | 20392 |
| Nefopam | 4450 | Lux Cellulose-4 | 1.64 | 13.31 min | PO | MeOH/IPA (90:10) DEA (0.1%) | 20376 |
| Suprofen | 5359 | Lux Cellulose-3 | 1.31 | 7.06 min | NP | Hex/EtOH (60:40) FA (0.1%) | 20098 |
| Tramadol | 33741 | Lux Cellulose-1 | 1.13 | 5.86 min | RP | ACN/20 mM $NH_4HCO_3$ (50:50) DEA (0.1%) | 20240 |

ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, MeOH = Methanol, FA = Formic acid, DEA = Diethylamine

* To view the full application enter the App ID onto the search field on our website.

# APPLICATIONS

**Figure 2.** Representative chromatograms for the chiral separation of anti-inflammatory agents




## Conclusion

In this study, we described the chiral separation of a variety of anti-inflammatory agents using Lux polysaccharide-based chiral stationary phases. All enantiomeric separations reported showed selectivity greater than 1.1 with the retention time for the first enantiomer below 15 min. Those separations can be used not only for analytical but for preparative purposes since our phases are available in various preparative formats such as Axia™ packed preparative columns or bulk media.

## References

1. Chankvetadze, B. *J. Chromatogr. A* **2012**, *1269*, 26-51. (Review).
2. Ikai, T.; Okamoto, Y. *Chem. Rev.* **2009**, *109*, 6077-6101.
3. Francotte, E. J. *Chromatogr. A* **2001**, *906*, 379-397. (Review)
4. Miller L. J. *Chromatogr. A* **2012**, *1250*, 250. (Review).



## 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* |
| | | | | | | | 10/pk | 10/pk |
| 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* |
| | | | | | | 10/pk | 10/pk |
| 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‡ |
| | | | 3/pk |
| 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 |

† Inquire for Lux 10 µm Cellulose-1 and Cellulose-2 columns.
* SecurityGuard Analytical Cartridges require holder, Part No. : KJ0-4282
‡ SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-7220

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## Lux® Ordering Information *(cont'd)*

| 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♦** |
| | | | | | /ea | /ea |
| 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 Lux 10 µm Cellulose-1 and Cellulose-2 columns | | | | for ID: | 18–29 mm | 30–49 mm |

**PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8223
♦ PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8277

| 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


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guarantee

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

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