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APPLICATIONS

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# Chromatographic Enantioseparation of Racemic Fungicide Agents using Lux® Polysaccharide-Based Chiral Stationary Phases

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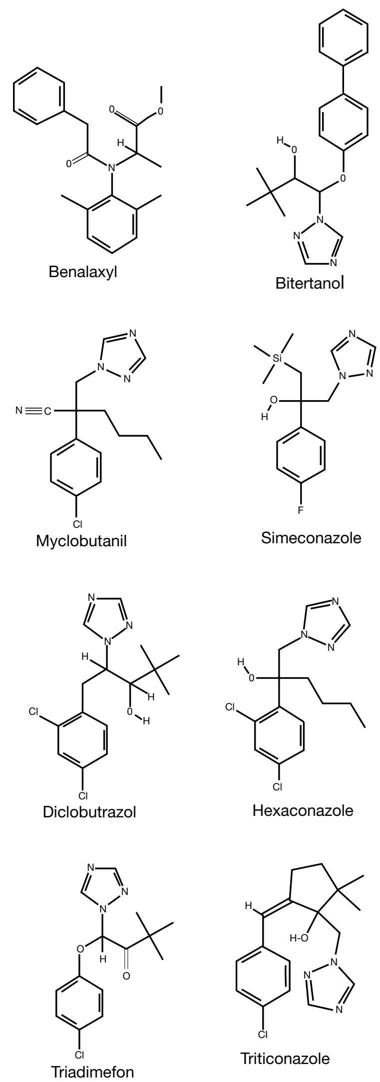
*In this technical note, we report the chiral chromatographic separation of various fungicide agents using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of Lux phases in normal phase and reversed phase separation modes.*

## Introduction

Fungicides have many positive uses such as increasing food production, decreasing damage to crops, reducing plant diseases, and more, but they also pose risks to humans and the environment. Of the 1693 pesticides listed in a recent review[1], 482 (28 %) are chiral (chemical compounds containing one or more centers of asymmetry) of which more than 80 are classified as fungicides. The degradation of those chiral fungicides by soil microbes is enantioselective[2] and each enantiomer will be eliminated from the environment following a different pathway. The degradation difference of chiral fungicides, combined with possible enantiospecific toxicity can affect not only efficacy, but also exposure and risk to humans and environment. In the pharmaceutical industry, mainly due to the potential enantiospecific toxicity, chiral drugs are routinely tested for chiral purity, whereas pesticides generally are not.

Separations of chiral compounds can be performed by chiral chromatography using chiral stationary phases (CSPs) in high performance liquid chromatography (HPLC). HPLC is recognized as the most popular and reliable tool for both analytical and preparative separation of chiral compounds. As a matter of fact, 76 % of the analytical chiral separations reported in the recent chiral pesticides review[1] were performed by HPLC, gas chromatography (GC) was second with 18 % of the separations reported. Polysaccharide-based CSPs such as Lux are the most widely used phases for the chromatographic separation of enantiomers.[3,4] Those CSPs show excellent success rate for chiral separation of a broad range of chiral compounds, as well as high loading ability for preparative applications. The various fungicide agents analyzed in this study are depicted in **Figure 1**. The chiral separations described in this application are the results of a systematic screening of five Lux polysaccharide-based CSPs (Cellulose-1, Cellulose- 2, Cellulose-3, Cellulose-4, and Amylose-2) under normal phase and reversed phase separation modes.

**Figure 1.**
Representative chemical structure of fungicides agents.




## 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

Eighteen fungicide agents racemates listed in **Table 1** were analyzed on Lux polysaccharide-based CSPs (Cellulose-1, Cellulose-2, Cellulose-3, Cellulose-4, and Amylose-2) in normal phase (NP) 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. For each compounds 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 compounds of this type. All the fungicides agents 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 at (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 4 and 15 min and all the separations are completed in less than 23 min. All the fungicides tested were basic or neutral and 0.1 % of diethylamine (DEA) was used as an additive. The presence of DEA favors dissociation of the amino group and improves peak shape.

**Table 1.**
Chiral separations of fungicides agents using Lux® polysaccharide-based CSPs

| Compound | CID | CSPs | ($\alpha$) | Rt(min) | Mode | Mobile Phase | App ID* |
|---|---|---|---|---|---|---|---|
| **Benalaxyl** | 51369 | Lux Cellulose-1 | 1.26 | 7.06 | NP | Hex/IPA (80:20) DEA (0.1 %) | 21665 |
| **Bitertanol** | 91656 | Lux Cellulose-3 | 1.52 | 8.96 | NP | Hex/EtOH (85:15) DEA (0.1 %) | 21670 |
| **Bromuconazole** | 3444 | Lux Cellulose-2 | 1.36 | 14.63 | RP | ACN/20 mM $NH_4HCO_3$ (60:40) DEA (0.1 %) | 21751 |
| **Cyproconazole** | 86132 | Lux Cellulose-2 | 1.23 | 7.08 | RP | ACN/20 mM $NH_4HCO_3$ (60:40) DEA (0.1 %) | 21754 |
| **Diclobutrazol** | 53309 | Lux Cellulose-1 | 1.27 | 4.50 | NP | Hex/IPA (80:20) DEA (0.1 %) | 21674 |
| **Difenoconazole** | 86173 | Lux Cellulose-3 | 1.07 | 11.07 | NP | Hex/EtOH (85:15) DEA (0.1 %) | 21681 |
| **Diniconazole** | 6436605 | Lux Cellulose-2 | 1.28 | 9.50 | RP | ACN/20 mM $NH_4HCO_3$ (60:40) DEA (0.1 %) | 21765 |
| **Epoxiconazole** | 3317081 | Lux Amylose-2 | 1.83 | 5.28 | RP | ACN/20 mM $NH_4HCO_3$ (60:40) DEA (0.1 %) | 21778 |
| **Flutriafol** | 91727 | Lux Cellulose-4 | 1.11 | 11.59 | RP | ACN/20 mM $NH_4HCO_3$ (40:60) DEA (0.1 %) | 21790 |
| **Hexaconazole** | 66461 | Lux Cellulose-4 | 1.52 | 8.22 | RP | ACN/20 mM $NH_4HCO_3$ (60:40) DEA (0.1 %) | 21802 |
| **Myclobutanil** | 6336 | Lux Cellulose-1 | 1.37 | 7.57 | RP | ACN/20 mM $NH_4HCO_3$ (60:40) DEA (0.1 %) | 21816 |
| **Paclobutrazol** | 616765 | Lux Amylose-2 | 1.37 | 4.51 | RP | ACN/20 mM $NH_4HCO_3$ (60:40) DEA (0.1 %) | 21829 |
| **Propiconazol** | 43234 | Lux Cellulose-1 | 1.33 | 6.83 | NP | Hex/IPA (80:20) DEA (0.1 %) | 21726 |
| **Simeconazole** | 10085783 | Lux Cellulose-2 | 1.70 | 6.72 | RP | ACN/20 mM $NH_4HCO_3$ (60:40) DEA (0.1 %) | 21830 |
| **Tebuconazole** | 86102 | Lux Cellulose-2 | 1.18 | 8.42 | RP | MeOH/20 mM $NH_4HCO_3$ (80:20) DEA (0.1 %) | 21836 |
| **Triadimefon** | 39385 | Lux Cellulose-2 | 1.30 | 6.09 | NP | Hex/IPA (80:20) DEA (0.1 %) | 21738 |
| **Triadimenol** | 41368 | Lux Cellulose-2 | 1.28 | 5.06 | NP | Hex/IPA (80:20) DEA (0.1 %) | 21739 |
| **Triticonazole** | 6436449 | Lux Cellulose-4 | 2.18 | 6.56 | RP | ACN/20 mM $NH_4HCO_3$ (70:30) DEA (0.1 %) | 21852 |

ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, $H_2O$ = Water, DEA = Diethylamine
* To view the full application enter the App ID onto the search field on our website.



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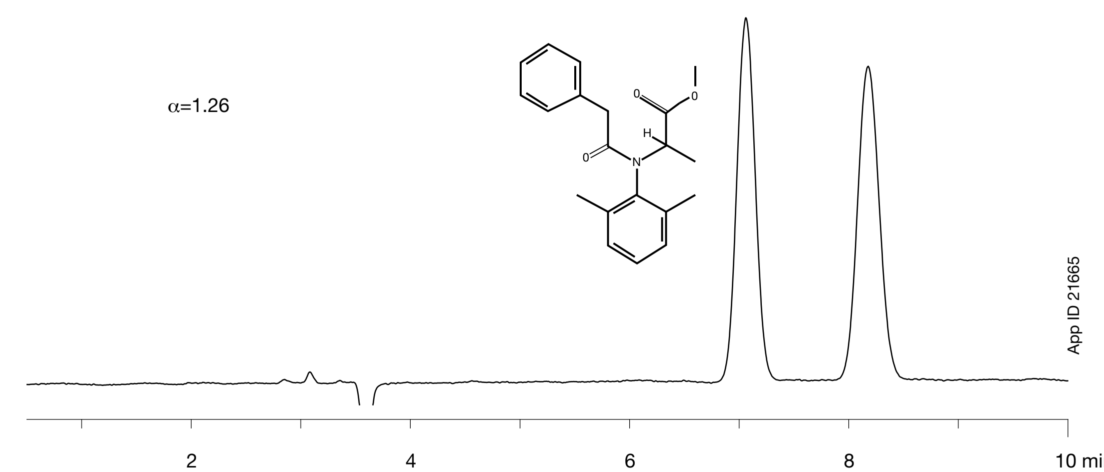
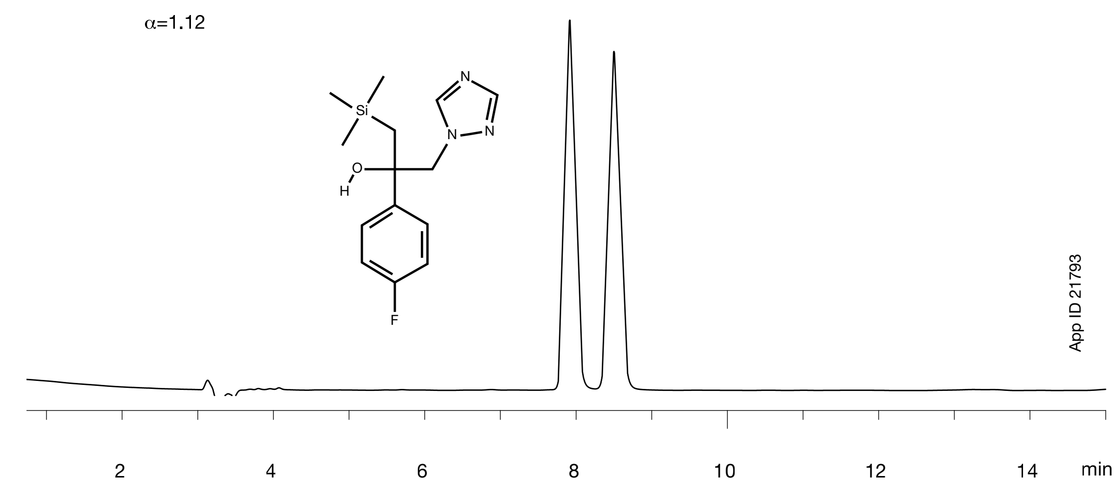
All of our Lux® products are pressure stable up to 300 bar. Two examples of chiral separation for Benalaxyl and Simeconazole are shown in **Figure 2**.

**Figure 2.**
Representative chromatograms for the chiral separation of fungicides

Benalaxyl on Lux Cellulose-1 in NP




Simeconazole on Lux Cellulose-2 in RP




## Conclusion

In this study, we described the chiral separation of a variety of fungicides 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. Ulrich E.M.; Morrison C.N.; Goldsmith M.R.; Foreman W.T. Reviews of Environmental Contamination and Toxicology, Springer, New York, NY, **2012**, 217, Chapter 1, 1-74.
2. Müller, M.D.; Buser, H.R. *Environ. Sci. Technol.* **1997**, 31, 1953–1959
3. Chankvetadze, B. *J. Chromatogr.* A **2012**, 1269, 26-51. (Review).
4. kai, T.; Okamoto, Y. *Chem. Rev.* **2009**, 109, 6077-6101.

APPLICATIONS

## Lux Ordering Information

| 3 µm Minibore, MidBore™, and Analytical Columns (mm) | | | | | | | | SecurityGuard™ Cartridges (mm) | |
|---|---|---|---|---|---|---|---|---|---|
| Phases | 50 x 2.0 | 150 x 2.0 | 150 x 3.0 | 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 | 00F-4458-Y0 | 00B-4458-E0 | 00D-4458-E0 | 00F-4458-E0 | 00G-4458-E0 | AJ0-8402 | AJ0-8403 |
| **Cellulose-2** | 00B-4456-B0 | 00F-4456-B0 | 00F-4456-Y0 | 00B-4456-E0 | 00D-4456-E0 | 00F-4456-E0 | 00G-4456-E0 | AJ0-8398 | AJ0-8366 |
| **Cellulose-3** | 00B-4492-B0 | 00F-4492-B0 | 00F-4492-Y0 | 00B-4492-E0 | 00D-4492-E0 | 00F-4492-E0 | 00G-4492-E0 | AJ0-8621 | AJ0-8622 |
| **Cellulose-4** | 00B-4490-B0 | 00F-4490-B0 | 00F-4490-Y0 | 00B-4490-E0 | 00D-4490-E0 | 00F-4490-E0 | 00G-4490-E0 | AJ0-8626 | AJ0-8627 |
| **Amylose-1** | 00B-4729-B0 | 00F-4729-B0 | 00F-4729-Y0 | 00B-4729-E0 | 00D-4729-E0 | 00F-4729-E0 | 00G-4729-E0 | AJ0-9337 | AJ0-9336 |
| **Amylose-2** | 00B-4471-B0 | 00F-4471-B0 | 00F-4471-Y0 | 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 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* |
| **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-1** | 00B-4732-B0 | 00B-4732-E0 | 00D-4732-E0 | 00F-4732-E0 | 00G-4732-E0 | AJ0-9337 | AJ0-9336 |
| **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 |

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

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