

APPLICATIONS

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

Marc Jacob, Michael Klein, and Allen Misa
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Allen Misa

**Industry
Marketing Manager**

Allen Misa is a downhill mountain biker who spends his days and weekends either riding off a face of a mountain or bouncing his 2 daughters on his knee.



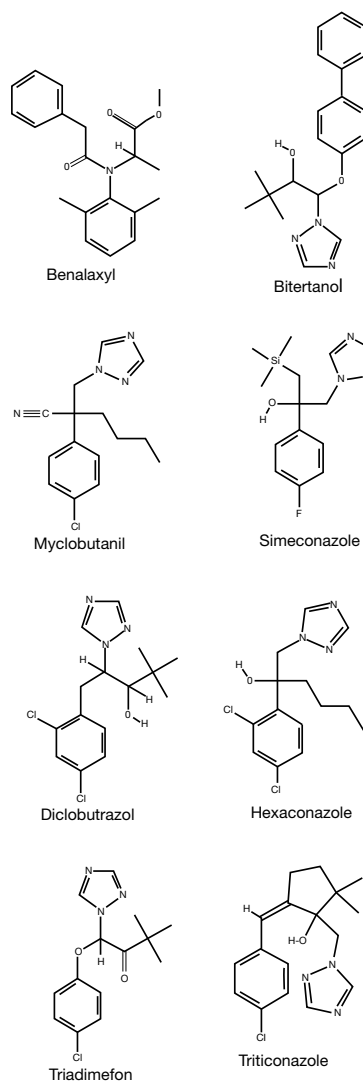
In this technical note, we report the chiral chromatographic separation of various fungicide agents using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of Lux phases in normal phase and reversed phase separation modes.

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 review¹, 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 enantioselective² 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 review¹ 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 4.6mm ID and all columns were packed with 5 μm particles. The flow rate was 1.0mL/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	(α)	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 ₄ HCO ₃ (60:40) DEA (0.1 %)	21751
Cyproconazole	86132	Lux Cellulose-2	1.23	7.08	RP	ACN/20 mM NH ₄ HCO 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 ₄ HCO ₃ (60:40) DEA (0.1 %)	21765
Epoxiconazole	3317081	Lux Amylose-2	1.83	5.28	RP	ACN/20 mM NH ₄ HCO ₃ (60:40) DEA (0.1 %)	21778
Flutriafol	91727	Lux Cellulose-4	1.11	11.59	RP	ACN/20 mM NH ₄ HCO ₃ (40:60) DEA (0.1 %)	21790
Hexaconazole	66461	Lux Cellulose-4	1.52	8.22	RP	ACN/20 mM NH ₄ HCO ₃ (60:40) DEA (0.1 %)	21802
Myclobutanil	6336	Lux Cellulose-1	1.37	7.57	RP	ACN/20 mM NH ₄ HCO ₃ (60:40) DEA (0.1 %)	21816
Pacllobutrazol	616765	Lux Amylose-2	1.37	4.51	RP	ACN/20 mM NH ₄ HCO 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 ₄ HCO ₃ (60:40) DEA (0.1 %)	21830
Tebuconazole	86102	Lux Cellulose-2	1.18	8.42	RP	MeOH/20 mM NH ₄ HCO 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 ₄ HCO ₃ (70:30) DEA (0.1 %)	21852

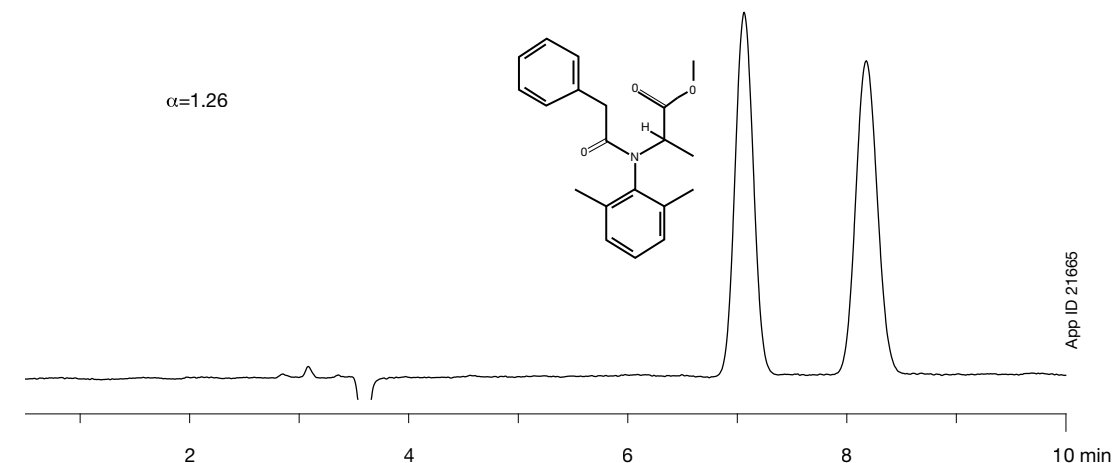
ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, H₂O = Water, DEA = Diethylamine
* To view the full application enter the App ID onto the search field on our website.

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**.

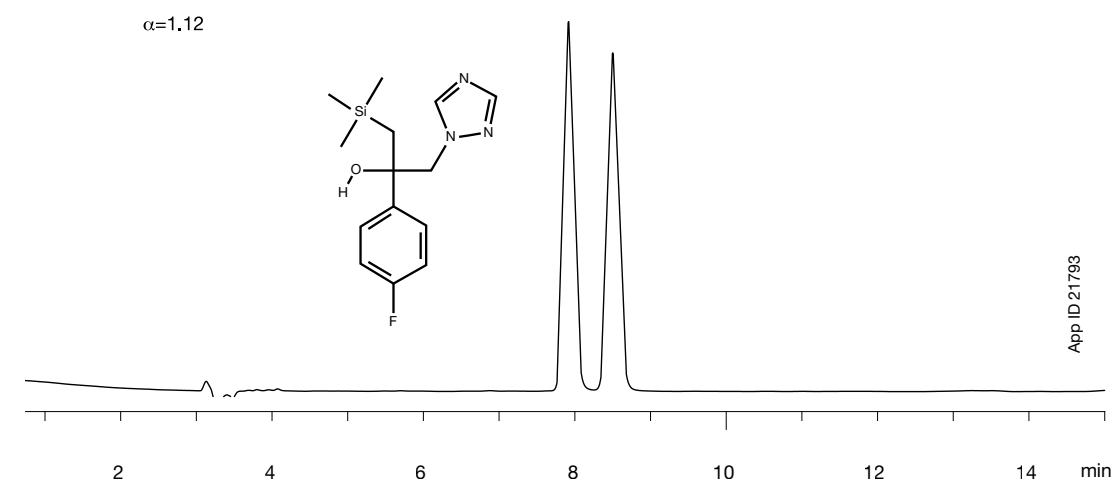
Figure 2.

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

- 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.
- Müller, M.D.; Buser, H.R. *Environ. Sci. Technol.* **1997**, 31, 1953-1959
- Chankvetadze, B. *J. Chromatogr. A* **2012**, 1269, 26-51. (Review).
- 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	AJO-8402	AJO-8403	
Cellulose-2	00B-4456-B0	00F-4456-B0	00F-4456-Y0	00B-4456-E0	00D-4456-E0	00F-4456-E0	00G-4456-E0	AJO-8398	AJO-8366	
Cellulose-3	00B-4492-B0	00F-4492-B0	00F-4492-Y0	00B-4492-E0	00D-4492-E0	00F-4492-E0	00G-4492-E0	AJO-8621	AJO-8622	
Cellulose-4	00B-4490-B0	00F-4490-B0	00F-4490-Y0	00B-4490-E0	00D-4490-E0	00F-4490-E0	00G-4490-E0	AJO-8626	AJO-8627	
Amylose-1	00B-4729-B0	00F-4729-B0	00F-4729-Y0	00B-4729-E0	00D-4729-E0	00F-4729-E0	00G-4729-E0	AJO-9337	AJO-9336	
Amylose-2	00B-4471-B0	00F-4471-B0	00F-4471-Y0	00B-4471-E0	00D-4471-E0	00F-4471-E0	00G-4471-E0	AJO-8471	AJO-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	AJO-8402	AJO-8403
Cellulose-2	00B-4457-B0	00B-4457-E0	00D-4457-E0	00F-4457-E0	00G-4457-E0	AJO-8398	AJO-8366
Cellulose-3	00B-4493-B0	00B-4493-E0	00D-4493-E0	00F-4493-E0	00G-4493-E0	AJO-8621	AJO-8622
Cellulose-4	00B-4491-B0	00B-4491-E0	00D-4491-E0	00F-4491-E0	00G-4491-E0	AJO-8626	AJO-8627
Amylose-1	00B-4732-B0	00B-4732-E0	00D-4732-E0	00F-4732-E0	00G-4732-E0	AJO-9337	AJO-9336
Amylose-2	00B-4472-B0	00B-4472-E0	00D-4472-E0	00F-4472-E0	00G-4472-E0	AJO-8471	AJO-8470

for ID: 2.0–3.0 mm 3.2–8.0 mm

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

Australia

t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China

t: +86 (0)20 2282-6668
f: +86 (0)20 2809-8130
chinainfo@phenomenex.com

Denmark

t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India

t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

Ireland

t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy

t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg

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

Mexico

t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands

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

New Zealand

t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico

t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain

t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com


United Kingdom

t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries

Corporate Office USA 
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com



If Lux analytical columns (≤ 4.6 mm ID) do not provide at least an equivalent or better chiral 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.

Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks

Lux is a registered trademark MidBore, Axia, and SecurityGuard are trademarks of Phenomenex. Agilent is a registered trademark of Agilent Technologies, Inc.

Disclaimer

Comparative separations may not be representative of all applications. Phenomenex is not affiliated with Agilent.

Axia column and packing technology is patented by Phenomenex. U.S. Patent No. 7,674,383 SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162,362

CAUTION: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or ULTRA holders, or to any cartridges.

© 2016 Phenomenex, Inc. All rights reserved.