Chromatographic Enantioseparation of Racemic Antifungal Drugs using Lux® Polysaccharide-Based Chiral Stationary Phases

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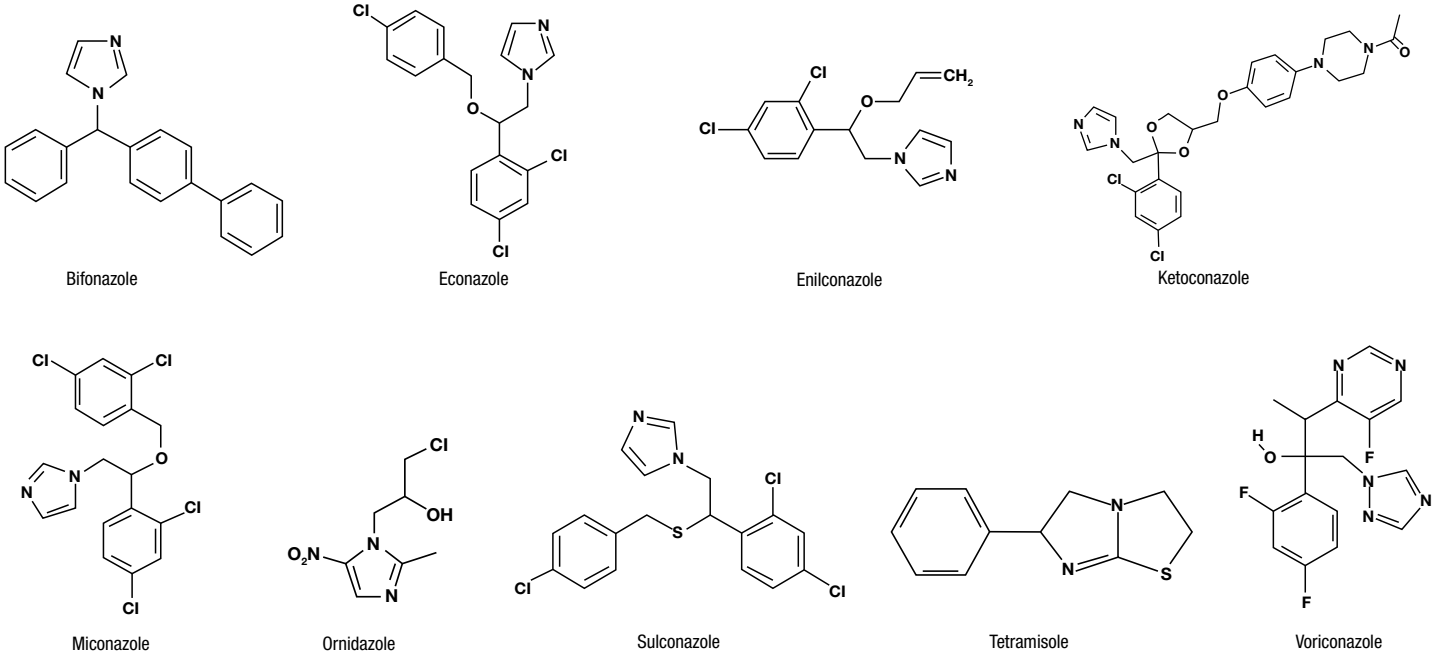
In this technical note, we report the chiral chromatographic separation of various antifungal agents using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase, polar organic, and reversed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

Imidazole and triazole antifungal drugs inhibit the enzyme responsible for converting lanosterol to ergosterol. Those drugs are effective in the treatment of fungal infections such as athlete's foot and ring worm. The various antifungal agents analyzed in this study are derived from imidazole or triazole and 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 structure of antifungal 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

Nine antifungal agents racemates 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 antifungal agents separated in this study are listed in **Table 1**. For each antifungal agents 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 antifungal 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 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 analytes such as antifungal agents, 0.1% of diethylamine (DEA) is used as mobile phase additive. DEA is an ion-masking agent that reduces unwanted interactions with residual silanols. DEA promotes improved peak shape by minimizing ion-exchange interactions between silanol groups and basic analytes. Interestingly, out of 9 separations, 7 are most successful in NP separation mode. NP mode is very similar in polarity and selectivity to supercritical fluid chromatography (SFC) mode. In SFC mode, ammonium hydroxide in MeOH, EtOH, or IPA can be used as basic additives to help peak shape.[4] SFC mode is particularly attractive for its high-throughput[5], low solvent consumption, low pressure drop, and high resolution. Another great advantage is the ease of scale–up to preparative scale, especially with our Axia™ packed preparative product line.

Table 1. Chiral separations of antifungal agents using Lux polysaccharide-based CSPs

Compound	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Bifonazole	2378	Lux Cellulose-2	1.57	8.9 min	NP	Hex/EtOH (80:20) DEA (0.1 %)	20506
Econazole	3198	Lux Cellulose-3	2.84	5.92 min	NP	Hex/EtOH (40/:60) DEA (0.1 %)	20110
Enilconazole	37175	Lux Cellulose-4	1.39	7.19 min	NP	Hex/IPA (60:40) DEA (0.1 %)	20427
Ketoconazole	3823	Lux Cellulose-1	1.25	13.71 min	PO	MeOH/IPA (90:10) DEA (0.1 %)	20353
Miconazole	4189	Lux Cellulose-3	2.18	5.21 min	NP	Hex/EtOH (96:4) DEA (0.1 %)	20129
Ornidazole	28061	Lux Amylose-2	5.36	5.46 min	NP	Hex/IPA (40:60) DEA (0.1 %)	20530
Sulconazole	5318	Lux Cellulose-2	1.67	12.25 min	NP	Hex/IPA (40:60) DEA (0.1 %)	20126
Tetramisole	3913	Lux Cellulose-2	1.48	6.66 min	PO	ACN/IPA (95:5) DEA (0.1 %)	20284
Voriconazole	5231054	Lux Cellulose-4	4.16	7.26 min	NP	Hex/EtOH (20:80) DEA (0.1 %)	20421

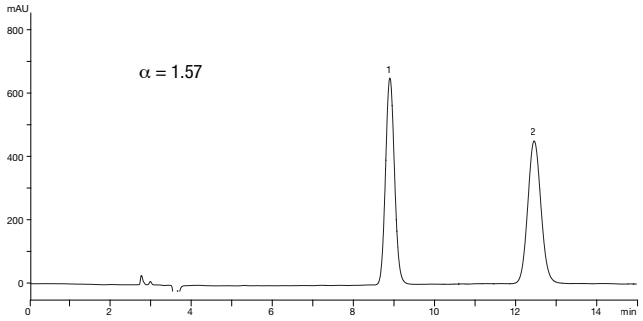
ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, MeOH = Methanol, FA = Formic acid, DEA = Diethylamine

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

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

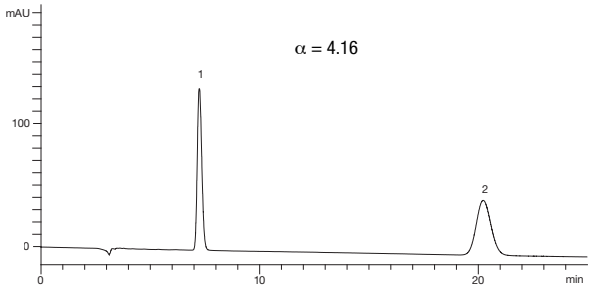
Figure 2. Representative chromatograms for the chiral separation of antifungal agents.

Bifonazole on Lux 5 µm Cellulose-2 in NP



App ID 20506

Voriconazole on Lux 5 µm Cellulose-4 in NP



App ID 20421

Conclusion

In this study, we described the chiral separation of a variety of antifungal 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 14 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.
4. Hamman, C.; Schmidt Jr., D. E.; Wong, M.; Hayes, M. J. *Chromatogr*. A **2011**, 1218, 7886– 7894.
5. Miller L. J. *Chromatogr*. A **2012**, 1250, 250. (Review).

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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* /10pk	4 x 3.0* /10pk
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* /10pk	4 x 3.0* /10pk
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‡ /3pk
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 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

APPLICATIONS

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
				for ID:	18–29 mm	30–49 mm

*Inquire for Lux 10 µm Cellulose-1 and Cellulose-2 columns

**HPLC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8223
SFC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8617

♦ HPLC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8277
SFC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8618

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

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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