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

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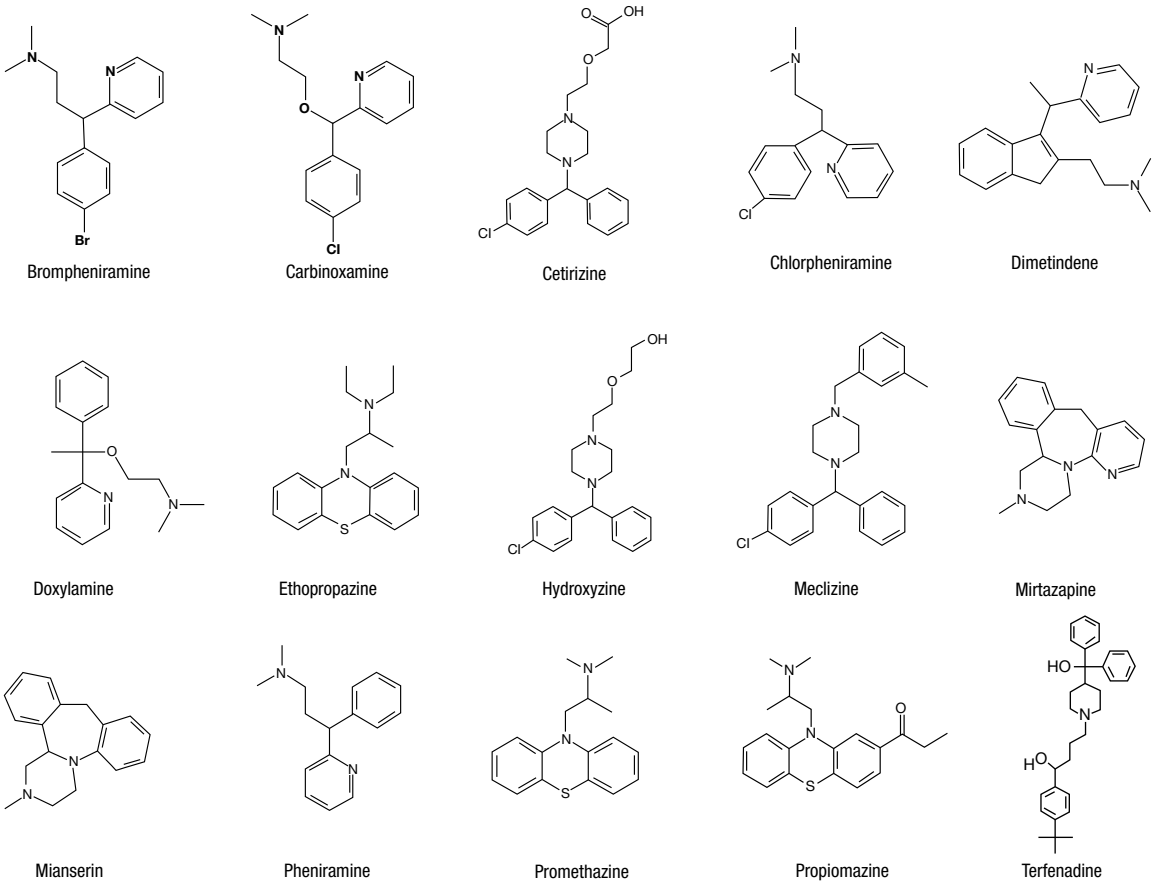
In this technical note, we report the chiral chromatographic separation of various anti-allergic drugs using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase, polar organic, and reversed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

Anti-allergic drugs, also known as histamine antagonists, are effective in the treatment of allergic reactions such as seasonal rhinitis and allergic dermatitis. The various anti-allergics 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 structure of anti-allergic drugs racemic mixtures



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

Fifteen anti-allergic 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 anti-allergic drugs separated in this study are listed in **Table 1**. For each anti-allergic 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-allergic drugs 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 10 min and all the separations are completed in less than 30 min. With basic analytes such as anti-allergics, 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 15 separations, 8 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 anti-allergic drugs using Lux polysaccharide-based CSPs

Beta Blocker	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Brompheniramine	6834	Lux Amylose-2	1.42	6.30	NP	Hex/IPA (90:10) DEA (0.1%)	20082
Carbinoxamine	2564	Lux Amylose-2	1.28	6.69	NP	Hex/EtOH (90:10) DEA (0.1%)	20452
Cetirizine	55182	Lux Cellulose-3	1.29	8.06	RP	ACN/20 mm NH_4HCO_3 (50:50) DEA (0.1%)	19641
Chlorpheniramine	2725	Lux Amylose-2	1.98	6.94	NP	Hex/EtOH (95:5) DEA (0.1%)	20445
Dimetindene	21855	Lux Cellulose-1	1.25	7.07	NP	Hex/EtOH (98:2) DEA (0.1%)	20435
Doxylamine	3162	Lux Cellulose-4	1.91	6.04	NP	Hex/IPA (90:10) DEA (0.1%)	20346
Ethopropazine	3290	Lux Cellulose-3	1.30	7.14	RP	MeOH/20 mm NH_4HCO_3 (95:5) DEA (0.1%)	20303
Hydroxyzine	3658	Lux Cellulose-3	1.66	7.54	RP	MeOH/20 mm NH_4HCO_3 (80:20) DEA (0.1%)	20320
Meclizine	4034	Lux Cellulose-3	2.62	5.52	NP	Hexane / EtOH (80:20) DEA (0.1%)	20338
Mianserin	4184	Lux Cellulose-1	1.25	8.14	RP	MeOH/20 mm NH4HCO3 (90:10) DEA (0.1%)	20225
Mirtazapine	4205	Lux Cellulose-2	1.32	5.80	PO	ACN/IPA (95:5) DEA (0.1%)	20067
Pheniramine	4761	Lux Cellulose-3	1.17	5.47	NP	Hexane/EtOH (95:5) DEA (0.1%)	20429
Promethazine	4927	Lux Cellulose-3	1.34	9.01	RP	MeOH/20 mm NH_4HCO_3 (95:5) DEA (0.1%)	20306
Propiomazine	4940	Lux Cellulose-3	1.37	5.18	PO	MeOH/IPA (90:10) DEA (0.1%)	20556
Terfenadine	5405	Lux Cellulose-2	1.30	7.00	NP	Hex/IPA (60:40) DEA (0.1%)	20078

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

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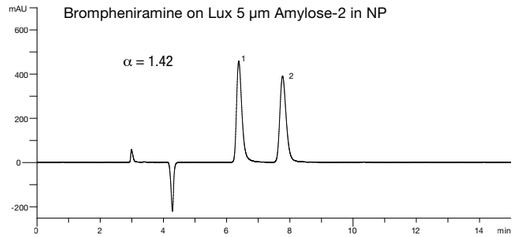
All of our Lux® products are pressure stable up to 300 bar and compatible with SFC separation mode using an organic modifier such as MeOH, EtOH, IPA, or ACN. Two examples of chiral separation for Brompheniramine and Ethopropazine are shown in **Figure 2**.

Figure 2. Representative chromatograms for the chiral separation of anti-allergics



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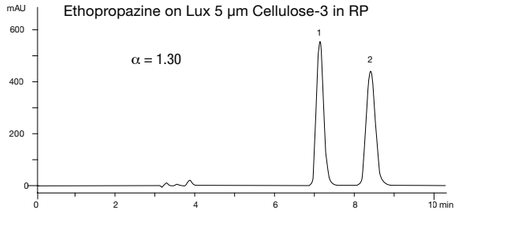

App ID 20303

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

Conclusion

In this study, we described the chiral separation of a variety of anti-allergic drugs 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 10 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 *(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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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.

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