Chiral Separation of Beta Blockers using Lux® Polysaccharide-Based Chiral Stationary Phases

Marc Jacob, Liming Peng, Michael McCoy, Michael Klein and Tivadar Farkas
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

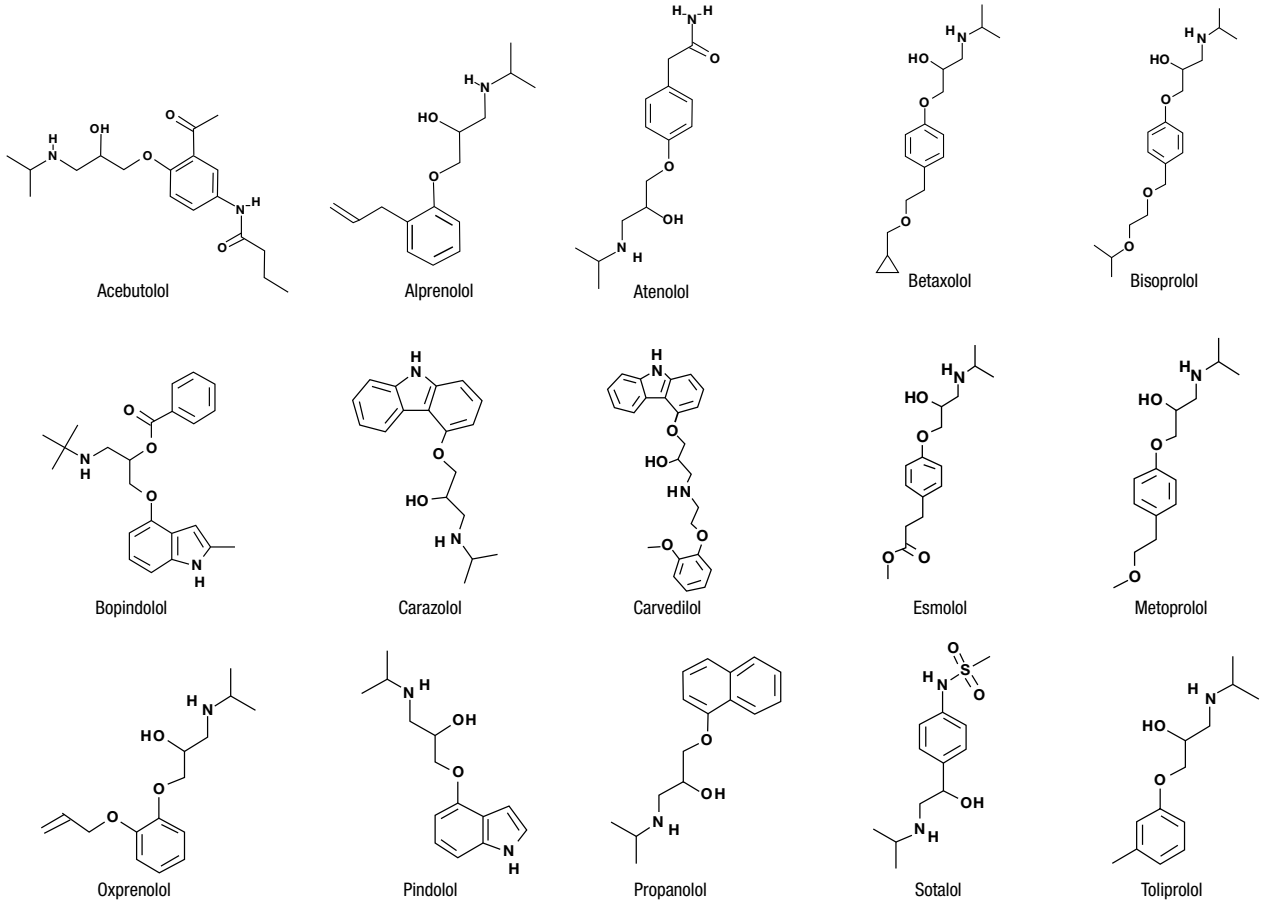
In this technical note, we report the chiral separation of various beta blocker pharmaceutical drugs using Lux polysaccharide-based chiral stationary phases. The reported separations are the results of a systematic screening of five different Lux phases in polar organic, normal phase, and reversed phase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

Beta blocker drugs, also known as beta adrenergic receptor antagonists, are effective in the treatment of cardiovascular diseases such as hypertension. The various beta blockers analyzed in this study are depicted in **Figure 1**. The chiral separations presented 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 15 Beta Blocker Racemates



APPLICATIONS

Material and Methods

All analyses were performed using an Agilent® 1100 series LC system (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with binary 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 beta blocker 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 beta adrenergic receptor antagonists analyzed in this study are listed in **Table 1**. For each beta blocker 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 beta blockers 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 4 and 15 min and all the separations are completed in less than 30 min. With amine derivatives such as beta blockers, we recommend to use 0.1 % of diethylamine (DEA) as an additive. The presence of DEA favors dissociation of the amino group and improves peak shape. Interestingly, out of 15 separations, 13 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, 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 Beta Blockers using Lux Polysaccharide-based CSPs

Beta Blocker	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Acebutolol	1978	Lux Amylose-2	4.21	4.91	PO	ACN/IPA (95:5) DEA (0.1 %)	18130
Alprenolol	2119	Lux Cellulose-2	1.12	6.12	NP	Hex/EtOH (95:5) DEA (0.1 %)	20443
Atenolol	2249	Lux Cellulose-1	1.39	10.55	NP	Hex/EtOH (80:20) DEA (0.1 %)	20547
Betaxolol	2369	Lux Cellulose-2	1.28	6.33	NP	Hex/EtOH (80:20) DEA (0.1 %)	20501
Bisoprolol	2405	Lux Cellulose-1	2.06	9.04	NP	Hex/EtOH (80:20) DEA (0.1 %)	20261
Bopindolol	44112	Lux Cellulose-4	1.22	5.03	RP	MeOH/20 mM NH_4HCO_3 (60:40) DEA (0.1 %)	20173
Carazolol	71739	Lux Cellulose-2	1.75	6.40	NP	Hex/IPA (70:30) DEA (0.1 %)	20117
Carvedilol	2585	Lux Cellulose-4	1.74	6.79	NP	Hex/IPA (40:60) DEA (0.1 %)	20422
Esmolol	59768	Lux Cellulose-1	2.04	6.10	NP	Hex/IPA (80:20) DEA (0.1 %)	20403
Metoprolol	4171	Lux Cellulose-1	1.97	5.27	NP	Hex/EtOH (80:20) DEA (0.1 %)	20470
Oxprenolol	4631	Lux Cellulose-1	3.09	5.25	NP	Hex/EtOH (80:20) DEA (0.1 %)	20544
Pindolol	4828	Lux Cellulose-2	2.13	10.39	NP	Hex/IPA (80:20) DEA (0.1 %)	20125
Propranolol	4946	Lux Cellulose-3	1.21	5.67	RP	MeOH/20 mM NH_4HCO_3 (80:20) DEA (0.1 %)	20308
Sotalol	5253	Lux Cellulose-2	1.29	14.19	NP	Hex/EtOH (90:10) DEA (0.1 %)	20550
Toliprolol	18047	Lux Amylose-2	1.17	5.97	NP	Hex/EtOH (90:10) DEA (0.1 %)	20511

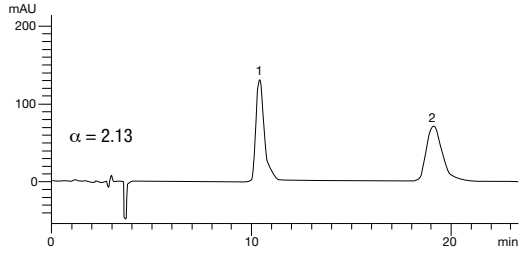
ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, MeOH = Methanol
* To view the full application enter the App ID onto the search field on our website.

APPLICATIONS

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 beta blockers pindolol and oxprenolol are shown in **Figure 2**.

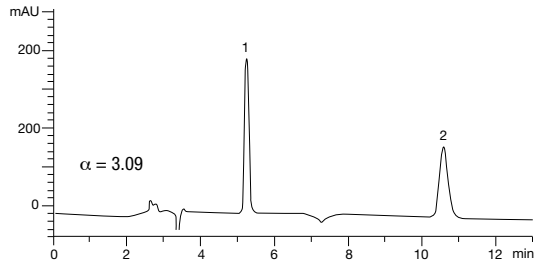
Figure 2.
Representative chromatograms for the separation of Beta Blockers

Pindolol on Lux 5 µm Cellulose-2 in NP



App ID 20125

Oxprenolol on Lux 5 µm Cellulose-1 in NP



App ID 20544

Conclusion

In this study, we described the chiral separation of a variety of beta blockers 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. *Chiral Separation Techniques: A Practical Approach*, 3rd ed.; Subramanian, G., Ed.; Wiley-VCH: Weinheim, Germany, **2007**.
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.

phenoLogix℠
Your Method. Our Scientists.

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
*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

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium
t: 02 503 4015 (French)
t: 02 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

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

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

Finland
t: 09 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

India
t: 040-3012 2400
f: 040-3012 2411
indiainfo@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

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

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

The Netherlands
t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

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

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

Sweden
t: 08 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

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

All other countries:
Corporate Office USA
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

phenomenex®
...breaking with tradition℠

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

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.

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

Trademarks
Lux is a registered trademark of Phenomenex. SecurityGuard and Axia 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 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.

TN9787111 2_W