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

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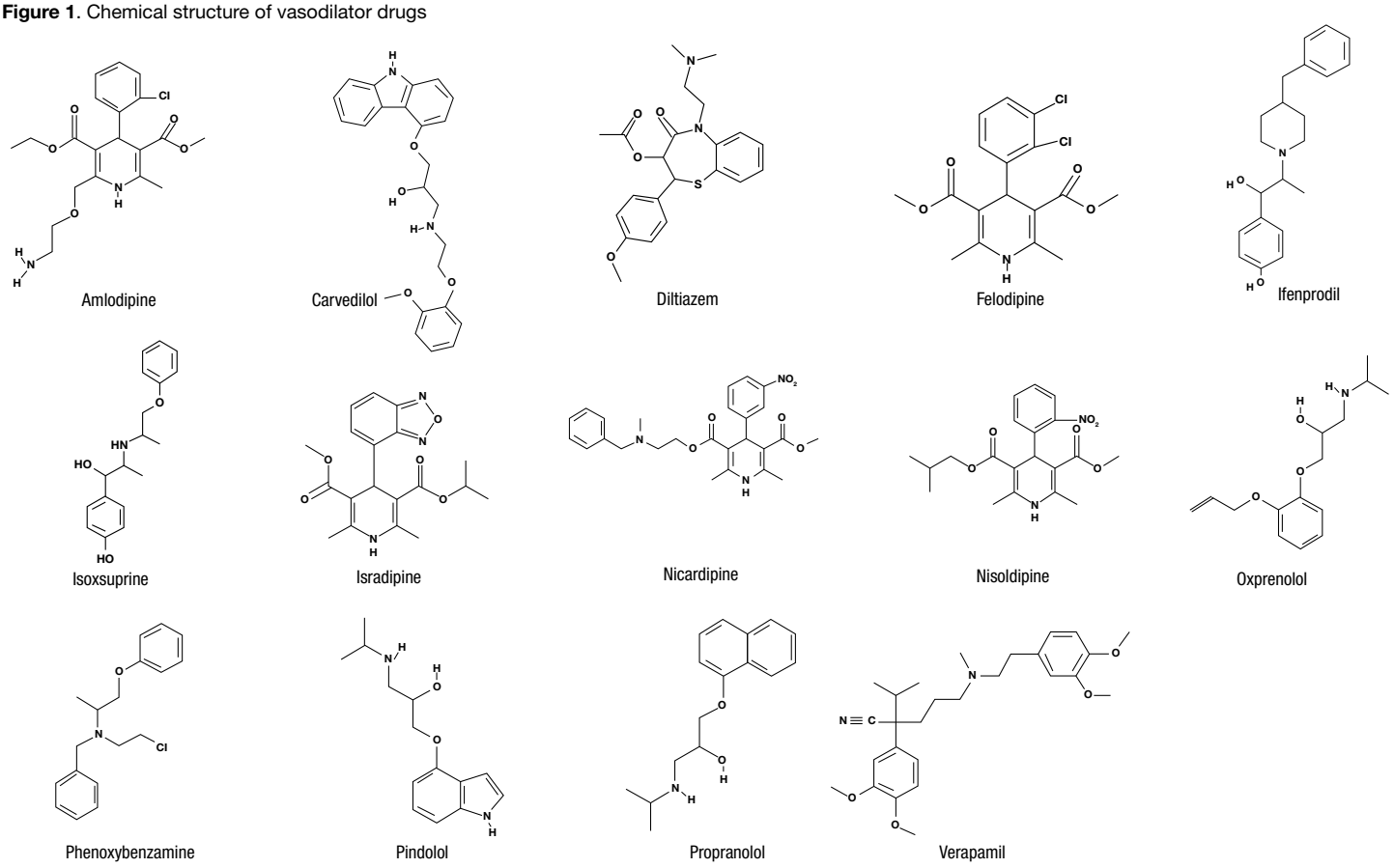
In this technical note, we report the chiral chromatographic separation of various vasodilator drugs using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase, polar organic, and reversed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

Vasodilator drugs are effective in the treatment of cardiovascular diseases such as hypertension, heart failure, and angina. The various vasodilator 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 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 vasodilator drugs



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

Fourteen vasodilator 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 vasodilator drugs separated in this study are listed in **Table 1**. For each vasodilator 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 vasodilator 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 19 min and all the separations are completed in less than 30 min. With basic analytes such as vasodilators, 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 14 separations, 10 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 vasodilator drugs using Lux polysaccharide-based CSPs

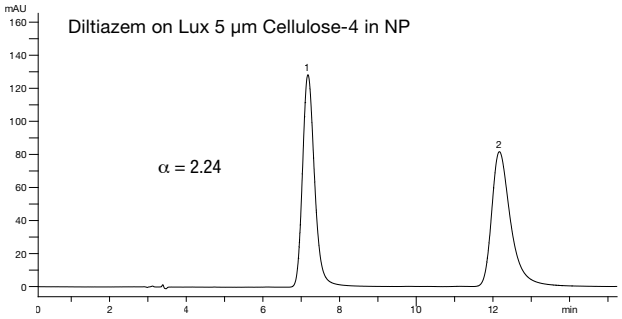
Compound	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Amlodipine	2162	Lux Cellulose-4	1.78	5.83 min	PO	ACN/IPA (95:5) DEA (0.1 %)	20358
Carvedilol	2585	Lux Cellulose-4	1.74	6.79 min	NP	Hex/IPA (40:60) DEA (0.1 %)	20422
Diltiazem	39186	Lux Cellulose-4	2.24	7.17 min	NP	Hex/IPA (60:40) DEA (0.1 %)	20458
Felodipine	3333	Lux Cellulose-3	1.26	10.73 min	RP	MeOH/20 mM NH_4HCO_3 (80:20) DEA (0.1 %)	20307
Ifenprodil	3689	Lux Amylose-2	1.44	6.21 min	NP	Hex/EtOH (80:20) DEA (0.1 %)	20517
Isoxsuprine	3783	Lux Cellulose-4	1.16	5.84 min	NP	Hex/EtOH (80:20) DEA (0.1 %)	20541
Isradipine	3784	Lux Amylose-2	1.13	9.9 min	NP	Hex/IPA (90:10) DEA (0.1 %)	20089
Nicardipine	4474	Lux Cellulose-1	1.13	18.9 min	NP	Hex/IPA (90:10) DEA (0.1 %)	20075
Nisoldipine	4499	Lux Cellulose-1	1.11	9.69 min	NP	Hex/IPA (90:10) DEA (0.1 %)	20276
Oxprenolol	4631	Lux Cellulose-1	3.09	5.25 min	NP	Hex/EtOH (80:20) DEA (0.1 %)	20544
Phenoxybenzamine	4768	Lux Cellulose-2	1.14	10.28 min	RP	MeOH/20 mM NH_4HCO_3 (80:20) DEA (0.1 %)	20233
Pindolol	4828	Lux Cellulose-1	1.99	5.16 min	RP	MeOH/20 mM NH_4Ac (80:20) DEA (0.1 %)	20198
Propranolol	4946	Lux Cellulose-1	1.35	6.9 min	NP	Hex/EtOH (80:20) DEA (0.1 %)	20477
Verapamil	2520	Lux Cellulose-3	1.38	6.25 min	NP	Hex/EtOH (60:40) DEA (0.1 %)	20114

ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, MeOH = Methanol, 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 Diltiazem and Propanolol are shown in **Figure 2**.

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



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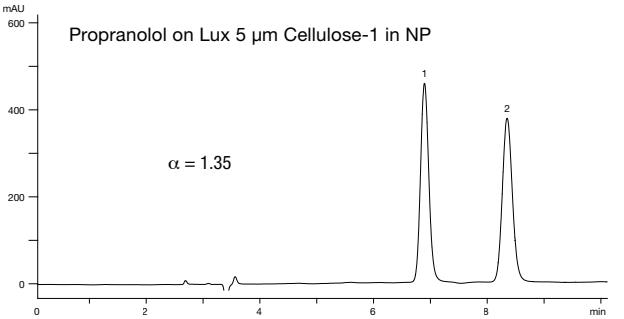

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Conclusion

In this study, we described the chiral separation of a variety of vasodilator 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 19 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

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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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