

APPLICATIONS

Chromatographic Enantioseparation of Racemic Antidepressive and Anti-Anxiety Drugs using Lux[®] Polysaccharide-Based Chiral Stationary Phases

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In this technical note, we report the chiral chromatographic separation of various antidepressive and anti-anxiety 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.

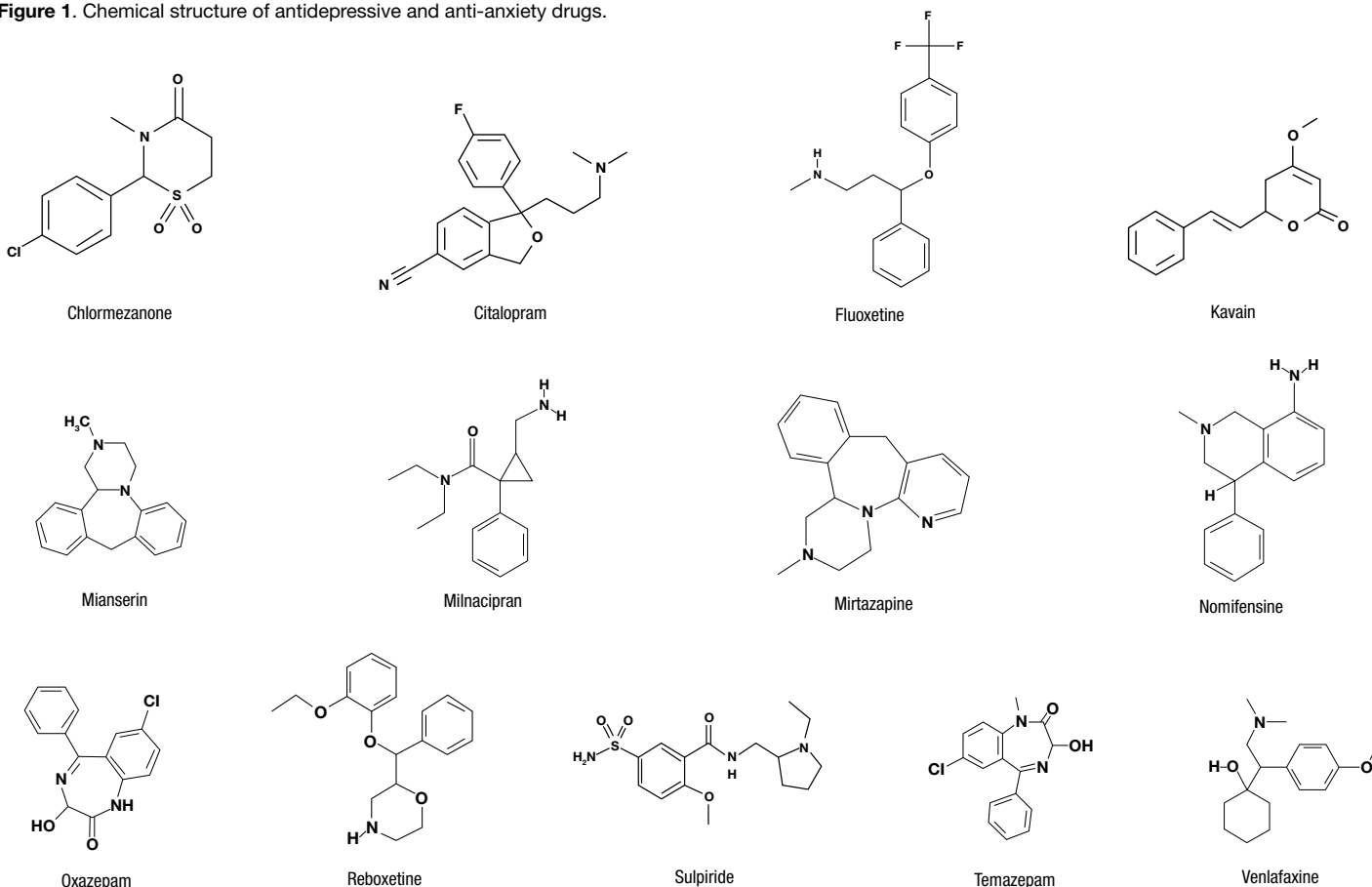
Introduction

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

Antidepressive and anti-anxiety drugs are used to treat various disorders such as depression, obsessive compulsive disorders, eating disorder or chronic pain and in some case insomnia. The various antidepressive and anti-anxiety drugs 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 antidepressive and anti-anxiety 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

Thirteen antidepressive and anti-anxiety 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 antidepressive and anti-anxiety drugs separated in this study are listed in **Table 1**. For each compound 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 antidepressive and anti-anxiety 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 12 min and all the separations are completed in less than 30 min. With basic analytes such as antidepressive and anti-anxiety drugs, 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.

Table 1. Chiral separations of antidepressive and anti-anxiety drugs using Lux polysaccharide-based CSPs

Compound	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Chlormezanone	2717	Lux Cellulose-3	1.36	5.3 min	PO	MeOH/IPA (90:10) DEA (0.1 %)	20371
Citalopram	2771	Lux Cellulose-4	1.41	9.11 min	NP	Hex/IPA (80:20) DEA (0.1 %)	20424
Fluoxetine	3386	Lux Cellulose-1	1.3	8.94 min	RP	MeOH/20 mM NH ₄ HCO ₃ (90:10) DEA (0.1 %)	20216
Kavain	5369129	Lux Cellulose-3	1.21	5.62 min	PO	MeOH/IPA (90:10) DEA (0.1%)	20365
Mianserin	4184	Lux Cellulose-1	1.25	8.14 min	RP	MeOH/20 mM NH ₄ HCO ₃ (90:10) DEA (0.1 %)	20225
Milnacipran	65833	Lux Cellulose-2	1.27	11.46 min	RP	MeOH/20 mM NH ₄ HCO ₃ (60:40) DEA (0.1 %)	20227
Mirtazapine	4205	Lux Cellulose-4	1.64	5.61 min	NP	Hex/IPA (80:20) DEA (0.1 %)	20425
Nomifensine	4528	Lux Cellulose-3	1.76	5.84 min	RP	MeOH/20 mM NH ₄ HCO ₃ (90:10) DEA (0.1 %)	20329
Oxazepam	4616	Lux Cellulose-1	2.32	5.94 min	RP	ACN/20 mM NH ₄ HCO ₃ (60:40) DEA (0.1 %)	20232
Reboxetine	3022645	Lux Cellulose-1	1.57	11.4 min	NP	Hex/IPA (80:20) DEA (0.1 %)	20056
Sulpiride	5355	Lux Cellulose-3	1.1	10.87 min	NP	Hex/EtOH (80:20) DEA (0.1 %)	20463
Temazepam	5391	Lux Cellulose-1	1.32	6.08 min	RP	ACN/20 mM NH ₄ HCO ₃ (60:40) DEA (0.1 %)	20236
Venlafaxine	5656	Lux Cellulose-2	1.11	6.47 min	NP	Hex/IPA (95:5) DEA (0.1 %)	20255

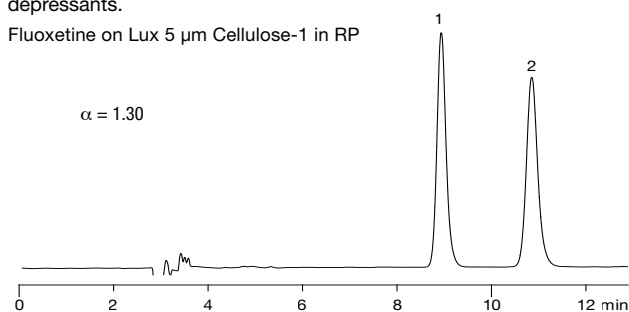
ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, MeOH = Methanol, DEA = Diethylamine

* 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 Fluoxetine and Milnacipran are shown in **Figure 2**.

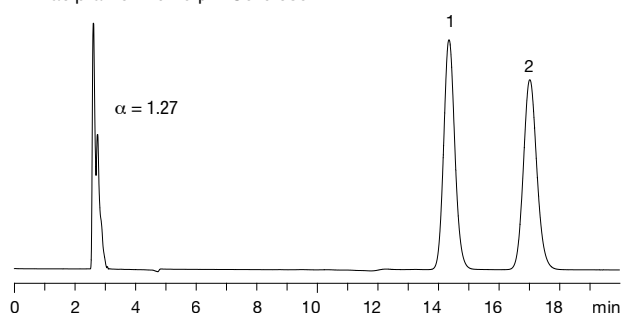
Figure 2. Representative chromatograms for the chiral separation of anti-depressants.

Fluoxetine on Lux 5 μ m Cellulose-1 in RP



App ID 20216

Milnacipran on Lux 5 μ m Cellulose-2 in RP



App ID 20227

Conclusion

In this study, we described the chiral separation of a variety of antidepressive and anti-anxiety 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 12 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.



Lux Ordering Information

3 μ m 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*	4 x 3.0*
							/10pk	/10pk
Cellulose-1	00B-4458-B0	00F-4458-B0	00B-4458-E0	00D-4458-E0	00F-4458-E0	00G-4458-E0	AJO-8402	AJO-8403
Cellulose-2	00B-4456-B0	00F-4456-B0	00B-4456-E0	00D-4456-E0	00F-4456-E0	00G-4456-E0	AJO-8398	AJO-8366
Cellulose-3	00B-4492-B0	00F-4492-B0	00B-4492-E0	00D-4492-E0	00F-4492-E0	00G-4492-E0	AJO-8621	AJO-8622
Cellulose-4	00B-4490-B0	00F-4490-B0	00B-4490-E0	00D-4490-E0	00F-4490-E0	00G-4490-E0	AJO-8626	AJO-8627
Amylose-2	00B-4471-B0	00F-4471-B0	00B-4471-E0	00D-4471-E0	00F-4471-E0	00G-4471-E0	AJO-8471	AJO-8470
							for ID: 2.0–3.0mm	3.2–8.0mm



5 μ m 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*
						/10pk	/10pk
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-2	00B-4472-B0	00B-4472-E0	00D-4472-E0	00F-4472-E0	00G-4472-E0	AJO-8471	AJO-8470
						for ID: 2.0–3.0mm	3.2–8.0mm

5 μ m 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	AJO-8404
Cellulose-2 [†]	00F-4457-N0	00G-4457-N0	AJO-8399
Cellulose-3	00F-4493-N0	00G-4493-N0	AJO-8623
Cellulose-4	00F-4491-N0	00G-4491-N0	AJO-8628
Amylose-2	00F-4472-N0	00G-4472-N0	AJO-8472
			for ID: 9–16mm

[†]Inquire for 10 μ m Cellulose-1 and Cellulose-2 columns.

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

[†]SemiPrep SecurityGuard[™] Cartridges require holder, Part No.: AJO-7220

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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-PO-AX	00G-4459-PO-AX	00G-4459-UO-AX	00G-4459-VO-AX	AJO-8405	AJO-8406
Cellulose-2*	00F-4457-PO-AX	00G-4457-PO-AX	00G-4457-UO-AX	00G-4457-VO-AX	AJO-8400	AJO-8401
Cellulose-3	00F-4493-PO-AX	00G-4493-PO-AX	00G-4493-UO-AX	00G-4493-VO-AX	AJO-8624	AJO-8625
Cellulose-4	00F-4491-PO-AX	00G-4491-PO-AX	00G-4491-UO-AX	00G-4491-VO-AX	AJO-8629	AJO-8630
Amylose-2	00F-4472-PO-AX	00G-4472-PO-AX	00G-4472-UO-AX	00G-4472-VO-AX	AJO-8473	AJO-8474

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

for ID:

18–29 mm

30–49 mm

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

* HPLC PREP SecurityGuard Cartridges require holder, Part No. : AJO-8277
SFC PREP SecurityGuard Cartridges require holder, Part No. : AJO-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



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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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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.

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