Screening Approach for the Separation of Pharmaceutical Compounds using Lux® Polysaccharide-Based Chiral Stationary Phases in SFC Mode

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In this technical note, we present various SFC screening approaches for the chiral separation of a 56-pharmaceutical compound test set using Lux polysaccharide-based chiral stationary phases. The new Lux Amylose-1 provides the largest number of baseline separations. Additional generic chiral separation strategies for SFC are highlighted for increasing success rates.

Introduction

Of the many techniques available for the separation of enantiomers, high performance liquid chromatography (HPLC) using polysaccharide-based chiral stationary phases (CSP) is currently the most popular.[1,2] Some of the reasons for this success include ease of use, high success rate, and ability to scale to preparative separations.[3]

However, over the past few years supercritical fluid chromatography (SFC) has regained interest as a valuable alternative chromatographic technique for chiral separations. The supercritical mobile phase, which typically constitutes a large percentage of carbon dioxide (>60 %), has a higher diffusivity and lower viscosity than liquid chromatography mobile phases. As a result, it is possible with SFC to run instruments at higher flow rates, which enables higher throughput by a reduction in column equilibration and analysis times. In addition, using SFC results in a lower consumption of organic solvent, thus decreasing costs and reducing environmental impact.[4]

With increasing workload and decreasing resources, fast and efficient chiral method development screening strategies are required to save development time. In this technical note, we report various screening approaches and results, derived from a representative group of 56 chiral pharmaceutical compounds (**Table 1**) using Lux polysaccharide-based CSPs (**Table 2**) under SFC conditions.

Most of the data summarized in this application has been extracted from an extended study performed by De Klerck *et al*.[5] from the Department of Analytical Chemistry and Pharmaceutical Technology of the Vrije Universiteit Brussel (Belgium). For a full breakdown of the results and explanations regarding the screening mobile phases, we recommend that the reader consults the article from this group as well as the references cited therein. The new Lux Amylose-1 phase was introduced by Phenomenex in September 2015 and the Amylose-1 screening results presented in this technote have not yet been published.

Table 1.
Fifty-six racemic pharmaceutical compounds screened in this study

Compounds	Compounds	Compounds
Acebutolol	*Flurbiprofen*	Oxazepam
Acenocoumarol	Hexobarbital	Oxprenolol
Alprenolol	*Ibuprofen*	Pindolol
Ambucetamide	Isothipendyl	Praziquantel
Atenolol	*Ketoprofen*	Procyclidine
Atropine	Labetalol	Promethazine
Betaxolol	*Mandelic acid*	Propiomazine
Bisoprolol	Mebeverine	Propranolol
Bopindolol	Mepindolol	Salbutamol
Bupranolol	Meptazinol	Salmeterol
Carazolol	Methadon	Sotalol
Carbinoxamine	Metoprolol	Sulpiride
Carvedilol	Mianserine	*Suprofen*
Clorphenamine	Nadolol	Terbutaline
Chlorthalidone	*Naringenin*	Tertatolol
Dimethindene	Nicardipine	Tetramisol
Ephedrine	Nimodipine	Verapamil
Esmolol	Nisoldipine	*Warfarin*
Fenoprofen	Nitrendipine	

Acidic compounds are written in italic
See TN17410515_W for table

Table 2.
Lux chiral phases used in this study

Column Name	Phase Description
Lux Amylose-1	Amylose tris(3,5-dimethylphenylcarbamate)
Lux Amylose-2	Amylose tris(5-chloro-2-methylphenylcarbamate)
Lux Cellulose-1	Cellulose tris(3,5-dimethylphenylcarbamate)
Lux Cellulose-2	Cellulose tris(3-chloro-4-methylphenylcarbamate)
Lux Cellulose-3	Cellulose tris(4-methylbenzoate)
Lux Cellulose-4	Cellulose tris(4-chloro-3-methylphenylcarbamate)

Material and Methods

Unless noted otherwise, the example analyses shown in this technote were performed using an analytical SFC method station from Thar® (Pittsburgh, PA, USA, a Waters® company) equipped with a Waters 2998-DAD detector (Milford, MA, USA) was used for the screening experiments. Data acquisition and processing were performed using ChromScope™ V1.10 software (2011) from Waters. The columns used for analysis were Lux Amylose-1, Amylose-2, Cellulose-1, Cellulose-2, Cellulose-3 and Cellulose-4 were obtained from Phenomenex (Torrance, CA, USA). All columns had dimensions 250 x 4.6 mm I.D. and 5 µm particle size. SFC conditions unless noted were the following: Flow Rate: 3 mL/min Temperature: 30 °C, Detection: UV @ 220 nm, Backpressure:150 bar, Injection Volume: 5 µL, Run Time: 30 min. Compounds that did not elute (entirely) within the set time frame of 30 minutes are considered as non-eluted. All solutions were prepared with sample concentrations of 0.5 mg/mL in methanol (MeOH). Pharmaceutical compounds and materials were purchased from various suppliers (see reference 5 for further details).

Results and Discussion

The test group of 56 racemic pharmaceutical compounds listed in **Table 1** was screened on all six commercially available Lux polysaccharide-based CSPs (Amylose-1, Amylose-2, Cellulose-1, Cellulose-2, Cellulose-3 and Cellulose-4) with eight different mobile phases under SFC conditions (**Table 3**). Methanol (MeOH) and Isopropanol (2PrOH) were each used as a co-solvent containing 2 additives with different concentrations, and used either separately or combined. These eight mobile phases have been identified by the Belgium group as a good compromise for highest chance of success with this representative set of 56 pharmaceutical compounds.

Table 3.
SFC mobile phases used in this study

MP	Description
A	CO_2/(MeOH with 0.5 % additive) 90/10
B	CO_2/(MeOH with 0.5 % additive) 80/20
C	CO_2/(MeOH with 0.25 % IPA and 0.25 % TFA) 90/10
D	CO_2/(MeOH with 0.1 % IPA and 0.1 % TFA) 80/20
E	CO_2/(2PrOH with 0.5 % additive) 90/10
F	CO_2/(2PrOH with 0.5 % additive) 80/20
G	CO_2/(2PrOH with 0.25 % IPA and 0.25 % TFA) 90/10
H	CO_2/(2PrOH with 0.1 % IPA and 0.1 % TFA) 80/20

MP = mobile phase, MeOH = methanol, 2PrOH = isopropanol/2-propanol, TFA = trifluoroacetic acid, IPA = isopropylamine. For acidic compounds, additive was TFA and for all other compounds (neutral, amphoteric, basic) IPA was used as additive.

The number of baseline separations (Rs > 1.5) for the six commercially available Lux CSPs under various mobile phase conditions are summarized in **Figure 1a** (MeOH as co-solvent) and **Figure 1b** (2PrOH as co-solvent). For this set of 56 pharmaceutical compounds, Lux Cellulose-1 and Lux Amylose-1 appear to be the most successful CSPs in term of baseline separations. Lux Cellulose-1 is the most successful with MeOH and Lux Amylose-1 is the most successful with 2PrOH as co-solvent. Lux Amylose-1 with mobile phase F (**Figure 1b**) provided the highest number of baseline separations with 32 compounds fully resolved out of 56 (57 % success rate).

Figure 1a.
Baseline separations using MeOH as co-solvent.

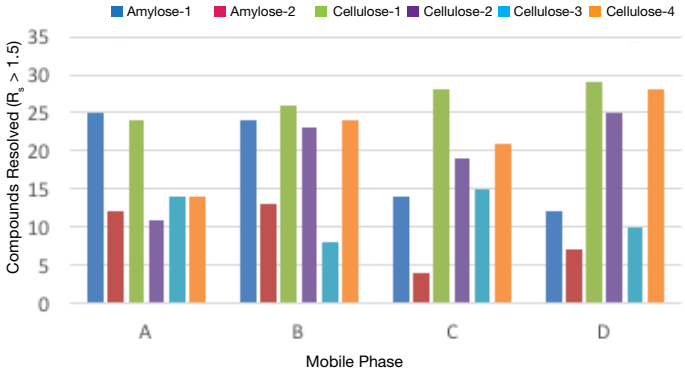
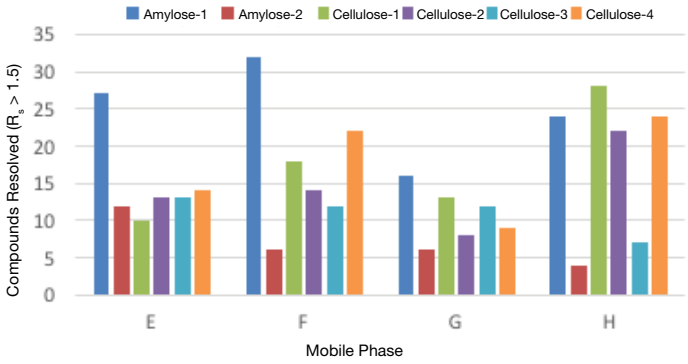


Figure 1b.
Baseline separations using 2PrOH as co-solvent.



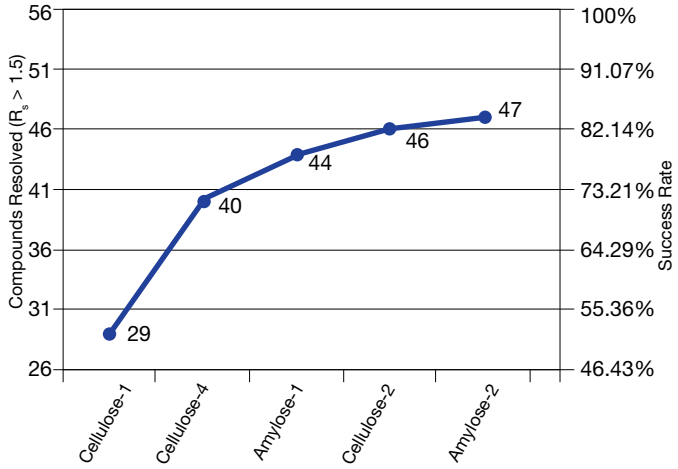
Screening Strategy

In order to find the most efficient screening conditions with our Lux CSPs, success rates were determined using one, two or multiple mobile phases with all six Lux CSPs. For the results with two mobile phases, we selected the screening mobile phases used in De Klerck *et al*.[5] A smaller resulting combination of columns/conditions means potentially an easier and faster route to finding a successful chiral separation.

Screening strategy using one mobile phase

In **Figure 2** the cumulative baseline separations for six Lux polysaccharide-based chiral columns using mobile phase D (20 % MeOH by volume as co-solvent with 0.1 % IPA and 0.1 % TFA as additives) are displayed. The baseline separations from the most successful CSP are recorded first, then the second CSP was selected based on the highest number of added unique baseline separations compared with the first, followed by the third, fourth, etc... Using this one mobile phase strategy, 47 of the 56 pharmaceutical compounds are baseline separated with 84 % success rate using mobile phase D and five CSPs. Lux Cellulose-3 does not generate additional separations so in fact only five CSP are required to obtain this success rate.

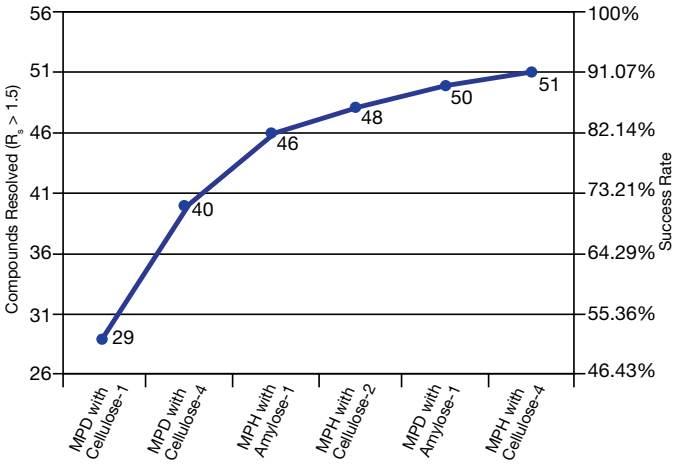
Figure 2.
Cumulative baseline separations with Lux phases using mobile phase D



Screening strategy using two mobile phases

In **Figure 3**, we looked at the cumulative baseline separations with Lux polysaccharide-based chiral columns using the two screening mobile phases of De Klerck *et al.*[5], being mobile phase D containing 20 volume % of MeOH as co-solvent and mobile phase H containing 20 % of 2PrOH as co-solvent. For both mobile phases, the additives used were 0.1 % IPA and 0.1 % TFA in the organic co-solvent. Using this two mobile phases strategy, 51 of the 56 pharmaceutical compounds were baseline resolved with 91 % success rate. In this case, only 4 columns are ultimately needed since Lux Amylose-2 and Cellulose-3 do not generate any additional unique separations.

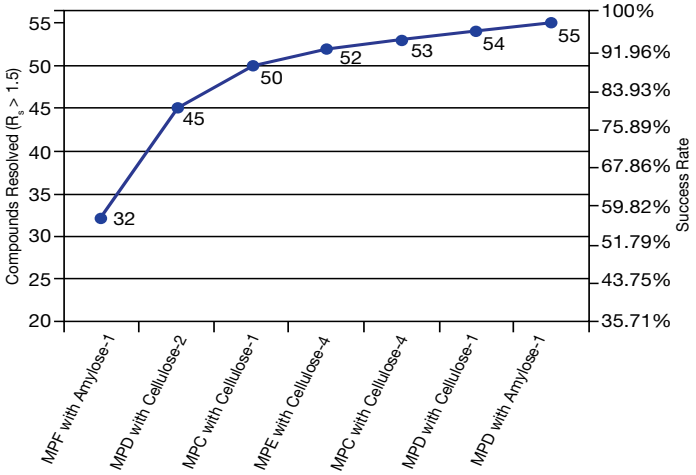
Figure 3.
Cumulative baseline separations with Lux phases using mobile phase D and H



Screening strategy using mulltiple mobile phases

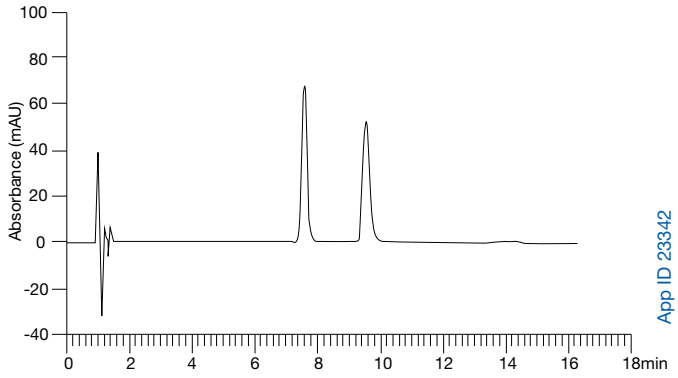
In order to find the most successful screening strategy with our Lux CSPs, the best combination for success rate was compiled using the minimum amount of Lux phases and mobile phases. This screening result is shown in **Figure 4**. By using 7 chromatographic systems, which require four mobile phases (C, D, E and F) and four Lux CSPs (Amylose-1, Cellulose-2, Cellulose-1 and Cellulose-4), 55 of the 56 compounds are baseline separated with an overall success rate of 98 %.

Figure 4.
Cumulative baseline separations across 7 chromatographic systems made up of four Lux phases and four mobile phases.



In **Figure 5**, we show a representative SFC chiral separation using mobile phase H with Lux Amylose-1. The chiral separations discussed in this application can be found on our website by typing the compound name into our application search portal www.phenomenex.com/Application/Search. The new applications on Lux Amylose-1 5 µm and 3 µm have also been recently added to our website.

Figure 5.
Chiral separation of Praziquantel by SFC



Column: Lux 5 µm Amylose-1
Dimensions: 250 x 4.6 mm
Part No.: 00G-4732-E0
Mobile Phase: CO_2/(2PrOH + 0.1 % IPA +0.1 %TFA) 80:20 (v/v) (H)
Flow Rate: 3 mL/min
Temperature: 30 °C

Conclusion

The results from this study clearly suggest the complexity of chiral screening under SFC conditions and the useful differences which can occur with changes in mobile phase composition. In particular the influence of additives on polysaccharide-based chiral stationary phases is yet to be fully understood. For the selected mixture of 56 racemic pharmaceutical compounds, we have demonstrated that screening with a well-selected mobile phase and four or five Lux polysaccharide-based CSPs can give a high probability of baseline separation. For those separations that are almost baseline resolved, selectivity and resolution can be positively adjusted by small changes in mobile phase composition. Additionally, 3 µm particle sizes are available for all six Lux CSPs to easily improve resolution by way of increasing efficiency.

References

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3. Francotte, E. *J. Chromatogr. A* **2001**, *906*, 379-397.
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APPLICATION

Lux® Ordering Information

3 µm Minibore, MidBore™, and Analytical Columns (mm)								SecurityGuard™ Cartridges (mm)	
Phases	**50 x 2.0**	**150 x 2.0**	**150 x 3.0**	**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	00F-4458-Y0	00B-4458-E0	00D-4458-E0	00F-4458-E0	00G-4458-E0	AJ0-8402	AJ0-8403
Cellulose-2	00B-4456-B0	00F-4456-B0	00F-4456-Y0	00B-4456-E0	00D-4456-E0	00F-4456-E0	00G-4456-E0	AJ0-8398	AJ0-8366
Cellulose-3	00B-4492-B0	00F-4492-B0	00F-4492-Y0	00B-4492-E0	00D-4492-E0	00F-4492-E0	00G-4492-E0	AJ0-8621	AJ0-8622
Cellulose-4	00B-4490-B0	00F-4490-B0	00F-4490-Y0	00B-4490-E0	00D-4490-E0	00F-4490-E0	00G-4490-E0	AJ0-8626	AJ0-8627
Amylose-1	00B-4729-B0	00F-4729-B0	00F-4729-Y0	00B-4729-E0	00D-4729-E0	00F-4729-E0	00G-4729-E0	AJ0-9337	AJ0-9336
Amylose-2	00B-4471-B0	00F-4471-B0	00F-4471-Y0	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 Minibore and 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	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-1	00B-4732-B0	00B-4732-E0	00D-4732-E0	00F-4732-E0	00G-4732-E0	AJ0-9337	AJ0-9336
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 Axia™ Packed Preparative Columns (mm)			SecurityGuard™ Cartridges (mm)
Phases	**150 x 21.2**	**250 x 21.2**	**15 x 21.2****
			/ea
Cellulose-1	00F-4459-P0-AX	00G-4459-P0-AX	AJ0-8405
Cellulose-2	00F-4457-P0-AX	00G-4457-P0-AX	AJ0-8400
Cellulose-3	00F-4493-P0-AX	00G-4493-P0-AX	AJ0-8624
Cellulose-4	00F-4491-P0-AX	00G-4491-P0-AX	AJ0-8629
Amylose-1	00F-4732-P0-AX	00G-4732-P0-AX	AJ0-9338
Amylose-2	00F-4472-P0-AX	00G-4472-P0-AX	AJ0-8473
		for ID:	18–29 mm

5 µm Axia™ Packed Preparative Columns (mm)			
Phases	**250 x 30**	**250 x 50**	**15 x 30.0***
			/ea
Cellulose-1	00G-4459-U0-AX	00G-4459-V0-AX	AJ0-8406
Cellulose-2	00G-4457-U0-AX	00G-4457-V0-AX	AJ0-8401
Cellulose-3	00G-4493-U0-AX	00G-4493-V0-AX	AJ0-8625
Cellulose-4	00G-4491-U0-AX	00G-4491-V0-AX	AJ0-8630
Amylose-1	00G-4732-U0-AX	00G-4732-V0-AX	AJ0-9339
Amylose-2	00G-4472-U0-AX	–	AJ0-8474
		for ID:	30–49 mm

* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282
**HPLC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223
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SFC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8618

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