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

Richard Hodgson[1], Michael Klein[2], Tom Cleveland[2], and Marc Jacob[2]
[1]Phenomenex LTD, Queens Avenue, Hurdsfield Industrial Estate, Macclesfield, Cheshire, SK10BN, United Kingdom
[2]Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

In this technical note, we report a novel screening approach for the chiral chromatographic separation, derived from a 56-pharmaceutical compound test set, using five Lux polysaccharide-based chiral stationary phases in supercritical fluid chromatography mode within an analysis time of 30 min or less.

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 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 is constituted of 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 to run instruments at higher flow rates, which enables higher throughput by a reduction in column equilibration and analysis times. In addition, SFC results in lowering consumption of organic solvent, decreasing costs, and reducing environmental impact.[4]

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

The results summarized in this application are extracted from an extended study performed by De Klerck *et al* [5]. For all results and explanations, we recommend the reader to consult the recent published article from this group as well as the references cited therein.

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

Material and Methods

The analyses shown in this technote were performed using an analytical SFC method station from Thar Instruments (Pittsburgh, PA, USA, a Waters® company) equipped with a Waters 2998-DAD detector (Milford, MA, USA). Data acquisition and processing were performed using ChromScope™ V1.10 software (2011) from Waters. The columns used for analysis: Lux Cellulose-1 (Cell-1), Cellulose-2 (Cell-2), Cellulose-3 (Cell-3), Cellulose-4 (Cell-4), and Amylose-2 (Amy-2) 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 otherwise 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 at sample concentration 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 five Lux® polysaccharide-based CSPs (Cellulose-1, Cellulose-2, Cellulose-3, Cellulose-4, and Amylose-2) with eight mobile phases under SFC conditions. The SFC mobile phases tested in this study are described in **Table 2**.

Table 2.
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) 90/10
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) with the five commercially available Lux CSPs are summarized in **Figure 1** for each mobile phase condition. For this set of 56 pharmaceutical compounds, Lux Cellulose-1, Cellulose-2, and Cellulose-4 returned the highest number of baseline separations for mobile phases B, D, F, and H. Lux Cellulose-1 showed the largest number of baseline separations for five of the eight mobile phases tested. The mobile phases showing the highest number of baseline separations were D and H. Both of these mobile phases contain a high concentration of organic solvent (20 %) and a combination of acidic (0.1 % TFA) and basic (0.1 % IPA) additives.

The increase in the number of baseline separations for mobile phases D and H is related to a decrease in retention time, resulting in elution (and separation) of a number of analytes that were eluting outside the 30 minute time window with weaker mobile phases. In addition, peaks were sharper when using higher modifier concentrations, resulting in slightly higher resolutions.

The cumulative baseline separations for the five Lux polysaccharide-based chiral columns using a mobile phase of CO_2/(MeOH with 0.1 % IPA and 0.1 % TFA), 80/20, v/v (D) is shown in **Figure 2**. Using this mobile phase, the baseline separations from the most successful CSP are recorded first. Then the second CSP is selected based on the highest number of added unique baseline separations compared with the first, followed by the third, fourth, and then fifth. Using this strategy, 46 of the 56 pharmaceutical compounds are baseline separated using mobile phase D and all five Lux CSPs.

Figure 2.
Cumulative baseline separations across five Lux phases using mobile phase of CO_2/(MeOH with 0.1 % IPA and 0.1 % TFA), 80/20 v/v

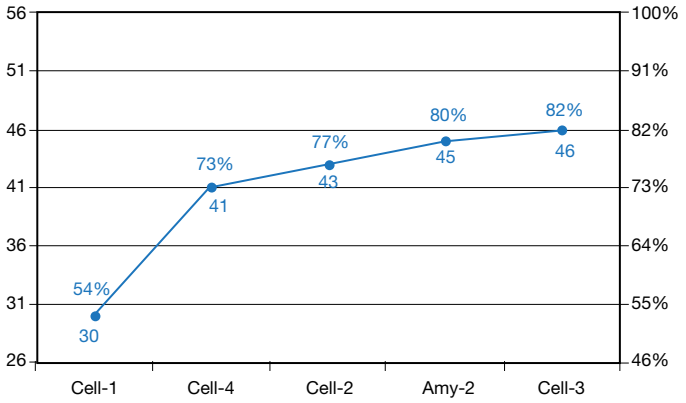
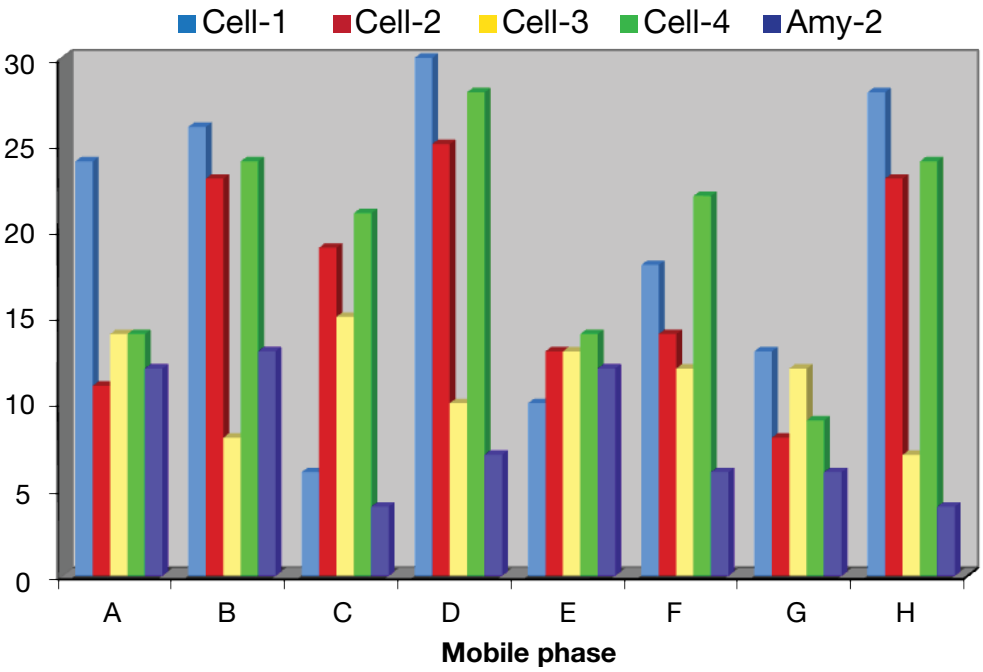
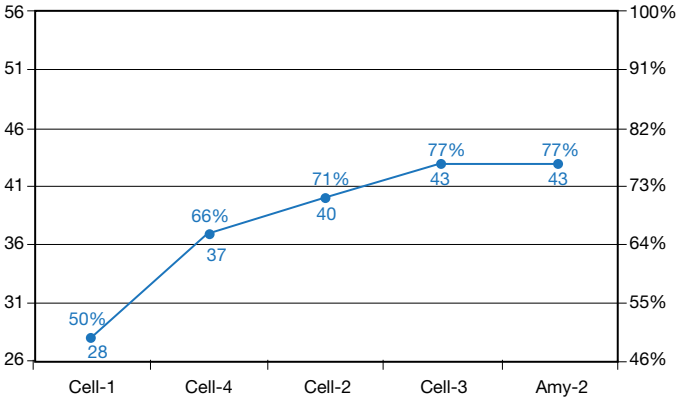


Figure 1.
Number of baseline separations for mobile phase A through H.



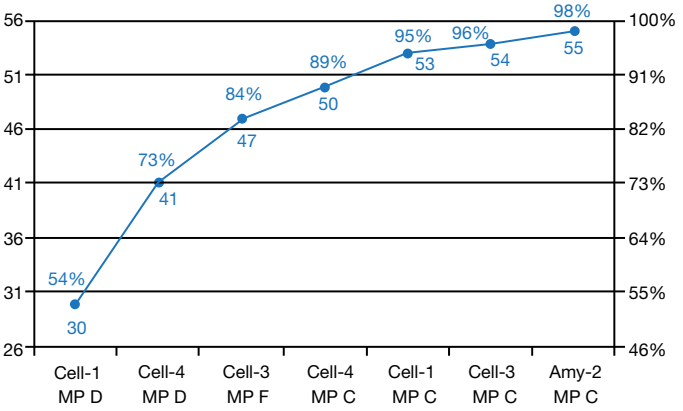
The cumulative baseline separations of the five Lux polysaccharide-based chiral columns using a mobile phase of CO_2/(2PrOH with 0.1 % IPA and 0.1 % TFA), 80/20, v/v (H) are shown in **Figure 3**. In the same way as before, 43 of the 56 pharmaceutical compounds are baseline resolved. In fact, only four CSPs are needed to obtain the maximal success rate.

Figure 3.
Cumulative baseline separations across five Lux® phases using mobile phase of CO_2/(2PrOH with 0.1 % IPA and 0.1 % TFA), 80/20 v/v (H)



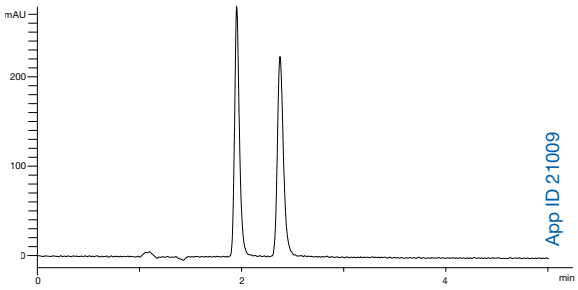
By using 7 chromatographic systems, which require three mobile phases (C, D, and F) and four Lux CSPs (Cellulose-1, Cellulose-3, Cellulose-4, and Amylose-2), 55 of the 56 test group compounds are baseline separated (**Figure 4**).

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



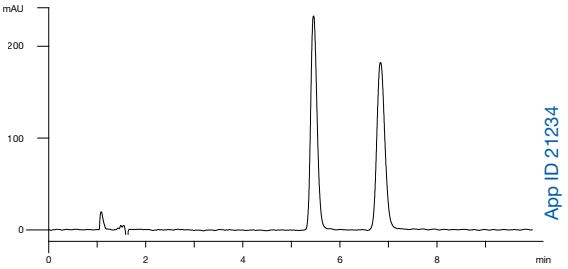
In **Figures 5a** and **5b**, some typical SFC separations are represented. All chiral separations with resolutions greater than 1.5 can be found on our application search webpage www.phenomenex.com/Application/Search.

Figure 5a.
Chiral separation of Alprenolol by SFC



Column: Lux 5u Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: CO_2/(Methanol + 0.5 % IPA) 80:20 (v/v) (B)
Flow Rate: 3 mL/min
Temperature: 30 °C

Figure 5b.
Chiral separation of Mianserine by SFC



Column: Lux 5u Cellulose-4
Dimensions: 250 x 4.6 mm
Mobile Phase: CO_2/(Methanol + 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 differences which can occur with relatively small changes in mobile phase composition. In particular, the influence of additives on the 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 single, well-selected mobile phase and four or five Lux polysaccharide based CSPs can give a high probability of baseline separation.

References

1. Chankvetadze, B. J. *Chromatogr. A* **2012**, *1269*, 26-51.
2. Ikai, T.; Okamoto, Y. Chem. Rev. **2009**, *109*, 6077-6101.
3. Francotte, E. J. *Chromatogr. A* **2001**, *906*, 379-397.
4. Miller L. J. *Chromatogr. A* **2012**, *1250*, 250.
5. De Klerck K.; Mangelings D.; Vander Heyden Y. *J. of Supercritical Fluids* **2013**, *80*, 50-59.

Acknowledgements

The authors would like to acknowledge K. De Klerck, D. Mangelings and Y. Vander Heyden (Department of Analytical Chemistry and Pharmaceutical Technology of the Vrije Universiteit Brussel (Belgium)) who allowed us to use data from reference 5.

APPLICATIONS

Lux® Ordering Information

3µm 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	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

5µm 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-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

*SecurityGuard Analytical Cartridges require holder, Part No. : KJ0-4282

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

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.

TN17411013_W