The Chiral Notebook

Multiple Solutions for Chiral Applications

You'll find expert advice on:

- Novel Chiral Column Screening Approach
- Chiral Method Development Tips
- Leveraging Easy Column Scale-up
- Chiral Resources

phenomenex®

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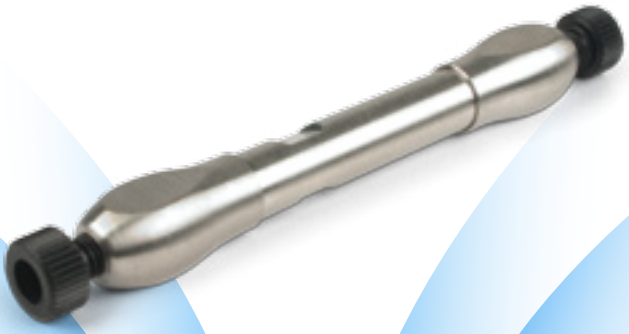
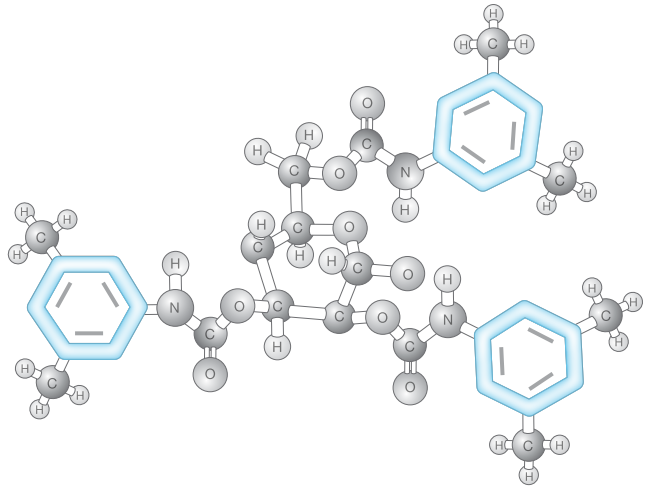


Table of Contents

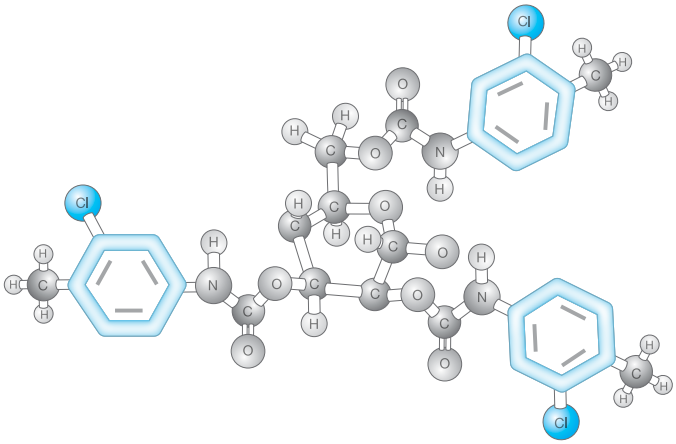
Alternative Selectivity of Chiral Stationary Phases Based on Cellulose tris(3-chloro-4-methylphenylcarbamate) and Cellulose tris(3,5-dimethylphenylcarbamate)

Liming Peng, Tivadar Farkas and Swapna Jayapalan
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

IntroductionPolysaccharide-based chiral stationary phases (CSP) are widely used due to their wide chiral recognition ability. Several cellulose and amylose derivatives are extremely effective in the separation of a wide range of compounds of interest in the pharmaceutical industry1. This work demonstrates the different chiral recognition capabilities of CSPs based on cellulose tris(3-chloro-4-methylphenylcarbamate) and cellulose tris(3,5-dimethylphenylcarbamate). Over 180 racemates of pharmaceutical interest were analyzed on these two phases in normal (NP), polar-organic (PO) and reversed phase (RP) separation modes. Numerous examples including important classes of drug compounds as well as statistical data prove that cellulose tris(3-chloro-4 methylphenylcarbamate) offers a good alternative to the commonly used cellulose tris(3,5- dimethylphenylcarbamate) in the separation of difficult racemic mixtures.Figure 1. Structures of Chiral Selective PhasesLux™ Cellulose-1Cellulose tris(3,5-dimethylphenylcarbamate)



Lux™ Cellulose-2 Cellulose tris(3-chlorlo-4-methyl-dimethylphenylcarbamate)



HPLC Conditions

Instrumentation	
HPLC System:	Agilent 1100 series (www.agilent.com)
Pump:	G1311A Quaternary Pump
Autosampler:	G1313A ALS
Detector:	G1315A Diode Array Detector

HPLC Conditions	
Flow Rate:	1.0 mL/min
Injection Volume:	5 - 20 µL (depending on analyte response)
Sample Concentration:	500 µg/mL racemate dissolved in mobile phase
Columns:	Lux™ 5 µm Cellulose-1; 250 x 4.6 mmLux™ 5 µm Cellulose-2; 250 x 4.6 mmCHIRALCEL® 5 µm OD-H®; 250 x 4.6 mm
Temperature:	Ambient
Detector:	UV @ 220 nm

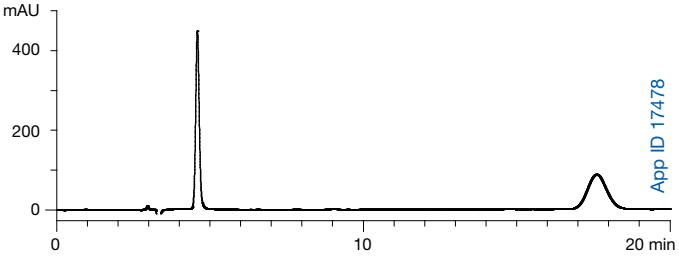
Table 1. Mobile Phase Compositions

Mobile Phase	NP	PO	
Basic andNeutralCompounds	0.1 % DEAin Hexane:IPA	0.1 % DEAin MeOH:IPA	0.1 % DEAin CH_3CN:IPA
Acidic andNeutralCompounds	0.1 % HAC(or FA) inHexane:IPA	0.1 % HAC(or FA) inMeOH:IPA	0.1 % HAC(or FA) inCH_3CN:IPA

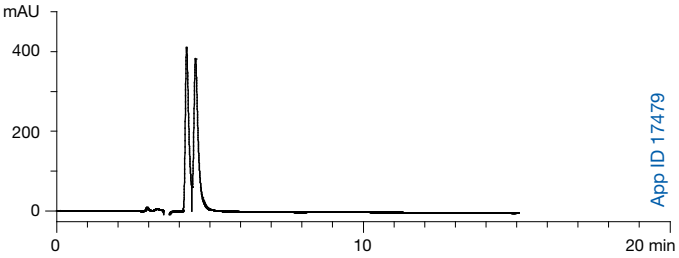
IPA: Iso-propanol; **DEA:** Diethylamine; **HAC:** Acetic Acid; **FA:** Formic acid; **MeOH:** Methanol; **CH_3CN:** Acetonitrile

Figure 2.
Enantioseparations of ß-Blockers in Normal Phase

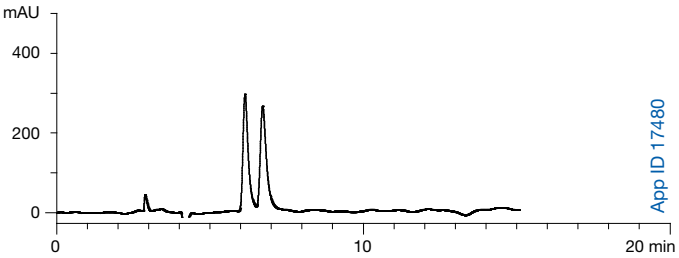
Toliprolol on Lux Cellulose-10.1 % DEA in Hexane / 0.1 % DEA in IPA (80:20)



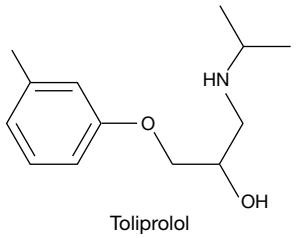
Toliprolol on Lux Cellulose-2 0.1 % DEA in Hexane / 0.1 % DEA in IPA (80:20)



Toliprolol on Lux Cellulose-2 0.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)

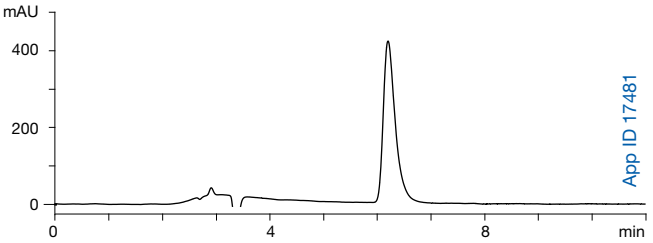


Column: Lux 5 µm Cellulose-1Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0 00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

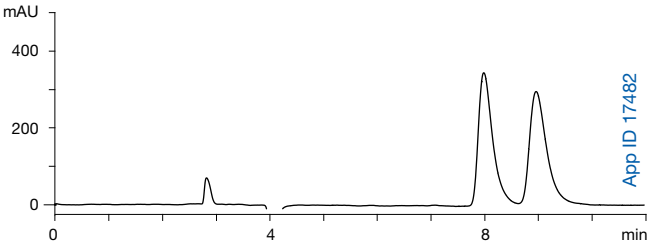


Toliprolol

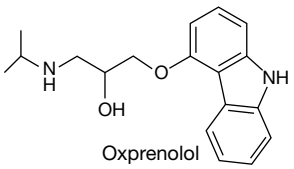
Oxprenolol on Lux Cellulose-10.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)



Oxprenolol on Lux Cellulose-20.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)

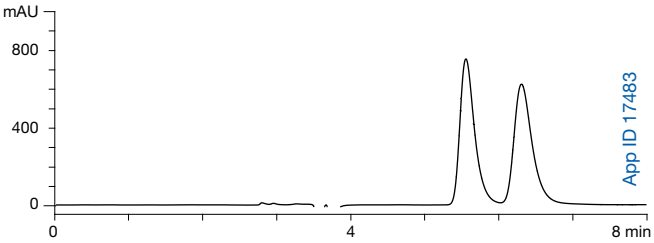


Column: Lux 5 µm Cellulose-1Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E000G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

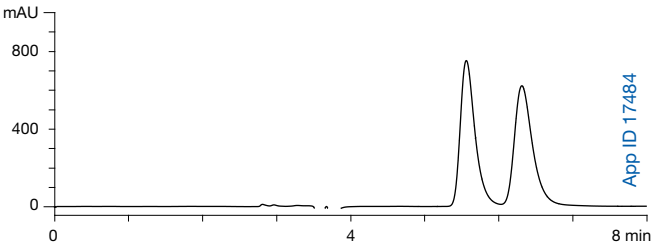


Oxprenolol

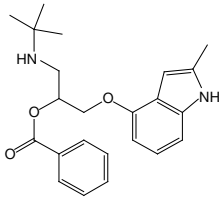
Bopindolol on Lux Cellulose-10.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)



Bopindolol on Lux Cellulose-20.1 % DEA in Hexane / 0.1 % DEA in IPA (90:10)



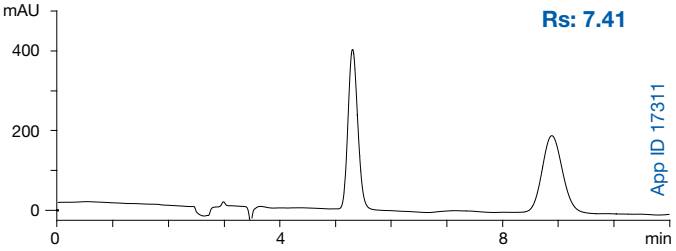
Column: Lux 5 µm Cellulose-1 Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E000G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



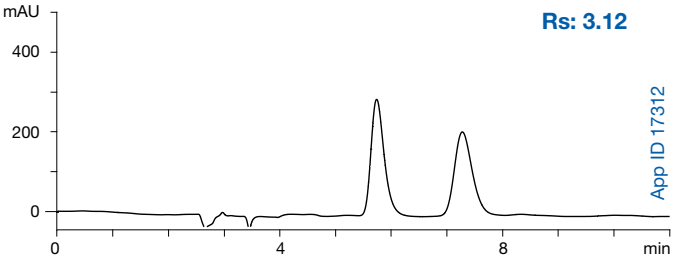
Bopindolol

Figure 3.
Enantioseparations in Normal Phase

Warfarin on Lux Cellulose-10.1 % Formic Acid in Hexane / 0.1 % Formic Acid in IPA (60:40)

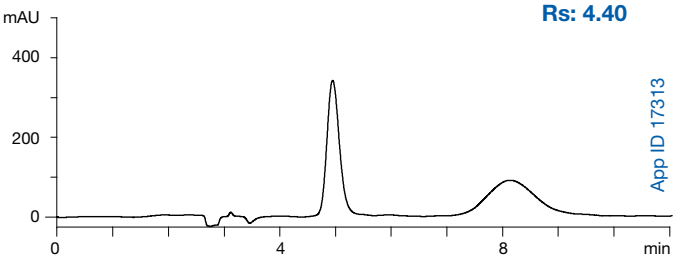


Warfarin on Lux Cellulose-20.1 % Formic Acid in Hexane / 0.1 % Formic Acid in IPA (60:40)

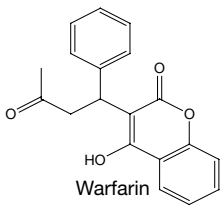


Column: Lux 5 µm Cellulose-1Lux
5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

Warfarin on CHIRALCEL®† OD-H®0.1 % Formic Acid in Hexane / 0.1 % Formic Acid in IPA (60:40)

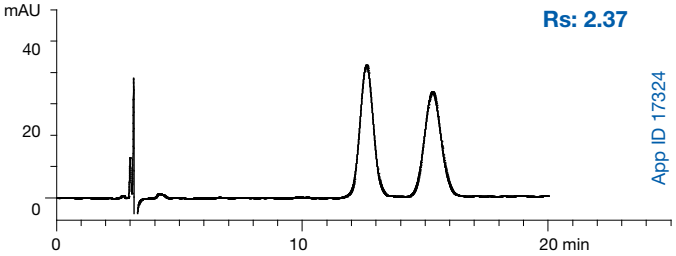


Column: CHIRALCEL® 5 µm OD-H®
Dimensions: 250 x 4.6 mm ID
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

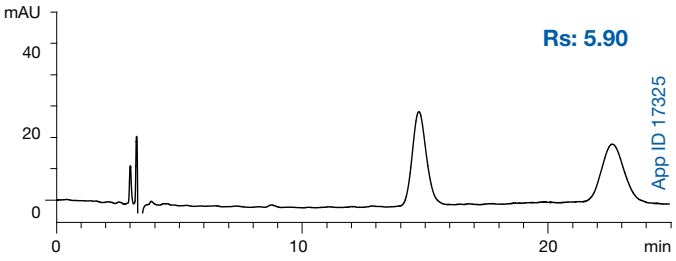


Warfarin

Sulcanozole on Lux Cellulose-10.1 % DEA in Hexane / 0.1 % DEA in IPA (60:40)

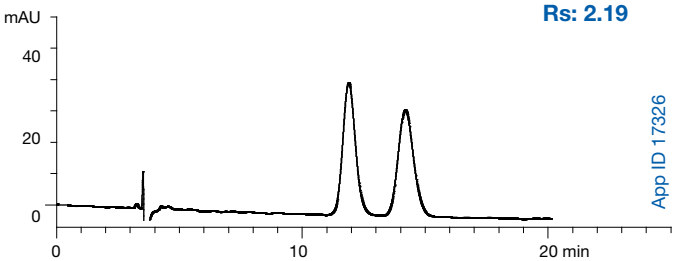


Sulcanozole on Lux Cellulose-20.1 % DEA in Hexane / 0.1 % DEA in IPA (60:40)

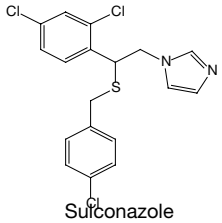


Column: Lux 5 µm Cellulose-1Lux
5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

Sulcanozole on CHIRALCEL®† OD-H®0.1 % DEA in Hexane / 0.1 % DEA in IPA (60:40)



Column: CHIRALCEL® 5 µm OD-H®
Dimensions: 250 x 4.6 mm ID
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

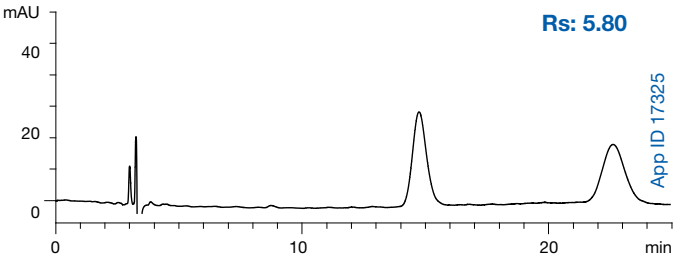


Sulconazole

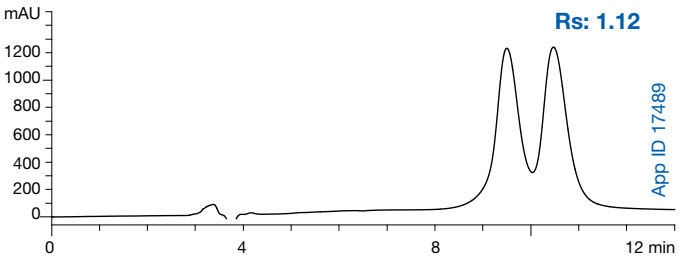
*with 0.1 % Formic Acid†CHIRALCEL and OD-H are registered trademarks of DAICEL Chemical Industries, Ltd. Comparative separations may not be representative of all applications.

Figure 4.
Complementary Enantioselectivity in Normal Phase and Polar-Organic

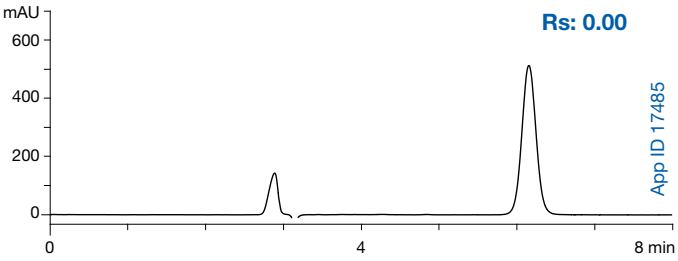
Sulcanozole on Lux Cellulose-2 0.1 % DEA in Hexane / 0.1 % DEA in IPA (60:40)



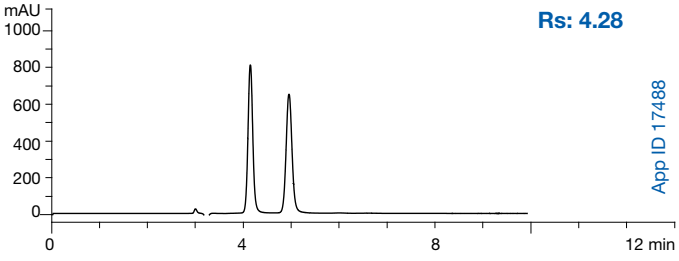
Milnacipran on Lux Cellulose-20.1 % DEA in Hexane / 0.1 % DEA in IPA (80:20)



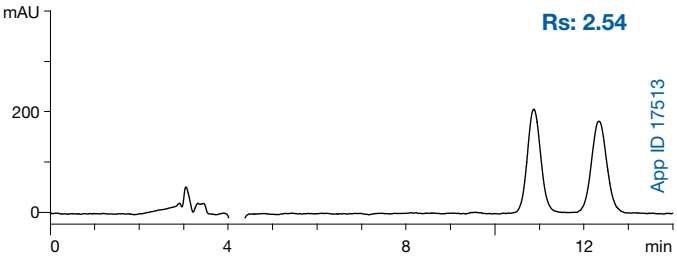
Sulcanozole on Lux Cellulose-2 0.1 % DEA in MeOH / 0.1 % DEA in IPA (95:5)



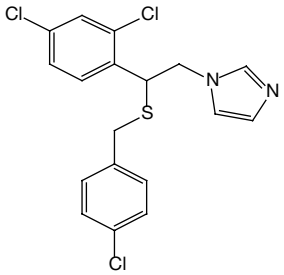
Milnacipran on Lux Cellulose-20.1 % DEA in MeOH / 0.1 % DEA in IPA (90:10)



Sulcanozole on Lux Cellulose-20.1 % DEA in CH_3CN / 0.1 % DEA in IPA (95:5)

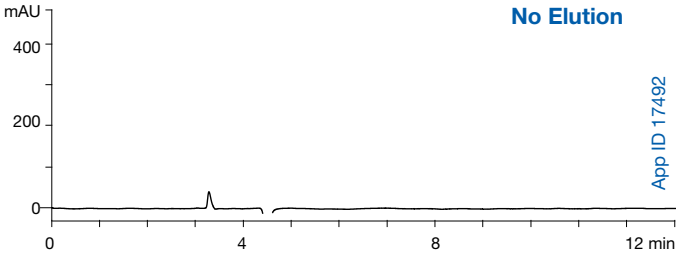


Column: Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient

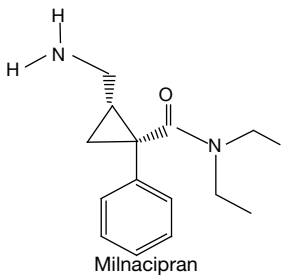


Sulconazole

Milnacipran on Lux Cellulose-20.1 % DEA in CH_3CN / 0.1 % DEA in IPA (95:5)



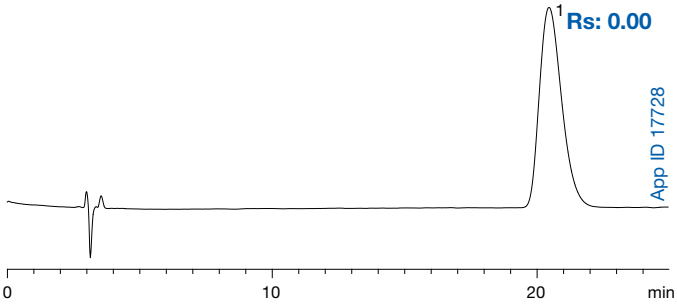
Column: Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



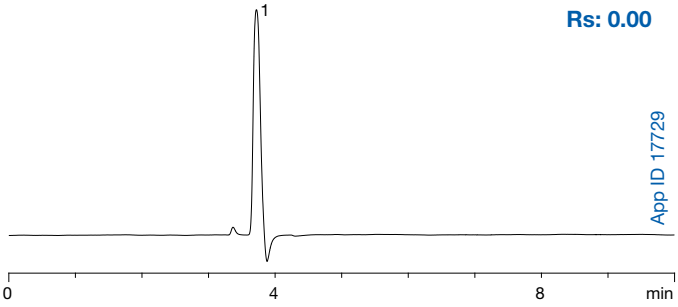
Milnacipran

Figure 4. (cont'd)
Complementary Enantioselectivity in Normal Phase and Polar-Organic

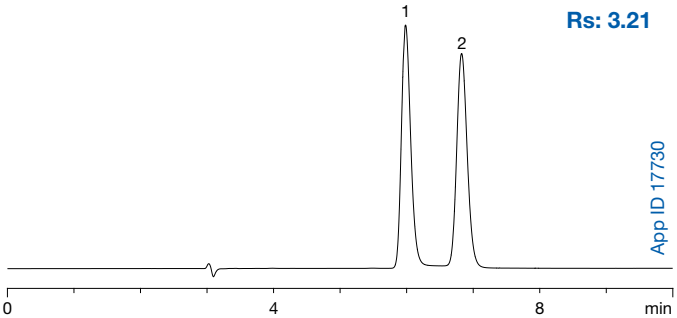
Chlormezanone on Lux Cellulose-10.1 % DEA in CH3CN / 0.1 % DEA in IPA (60:40)



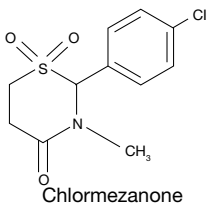
Chlormezanone on Lux Cellulose-10.1 % DEA in CH3CN / 0.1 % DEA in IPA (95:5)



Chlormezanone on Lux Cellulose-10.1 % DEA in MeOH / 0.1 % DEA in IPA (90:10)



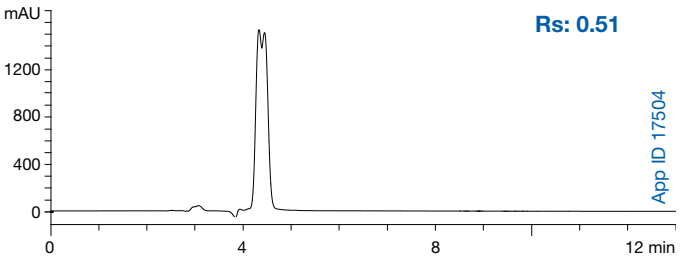
Column: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4459-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



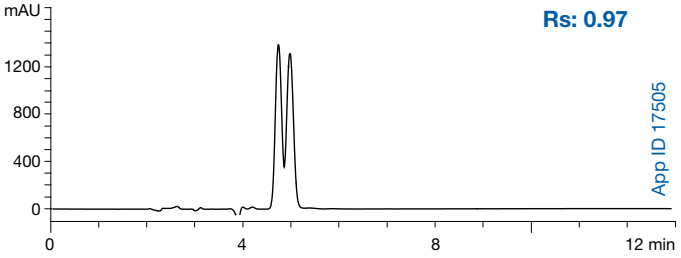
Chlormezanone

Figure 5.
Enantioseparations in Reversed Phase

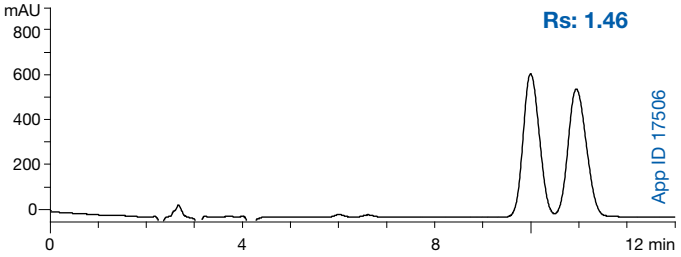
Clenbuterol on Lux Cellulose-20.1 % DEA in MeOH / 0.1 % DEA in Water (80:20)



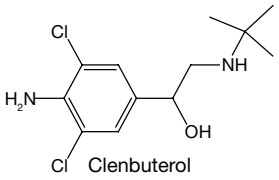
Clenbuterol on Lux Cellulose-20.1 % DEA in CH_3CN / 0.1 % DEA in Water (60:40)



Clenbuterol on Lux Cellulose-20.1 % DEA in CH_3CN / 0.1 % DEA in Water (40:60)



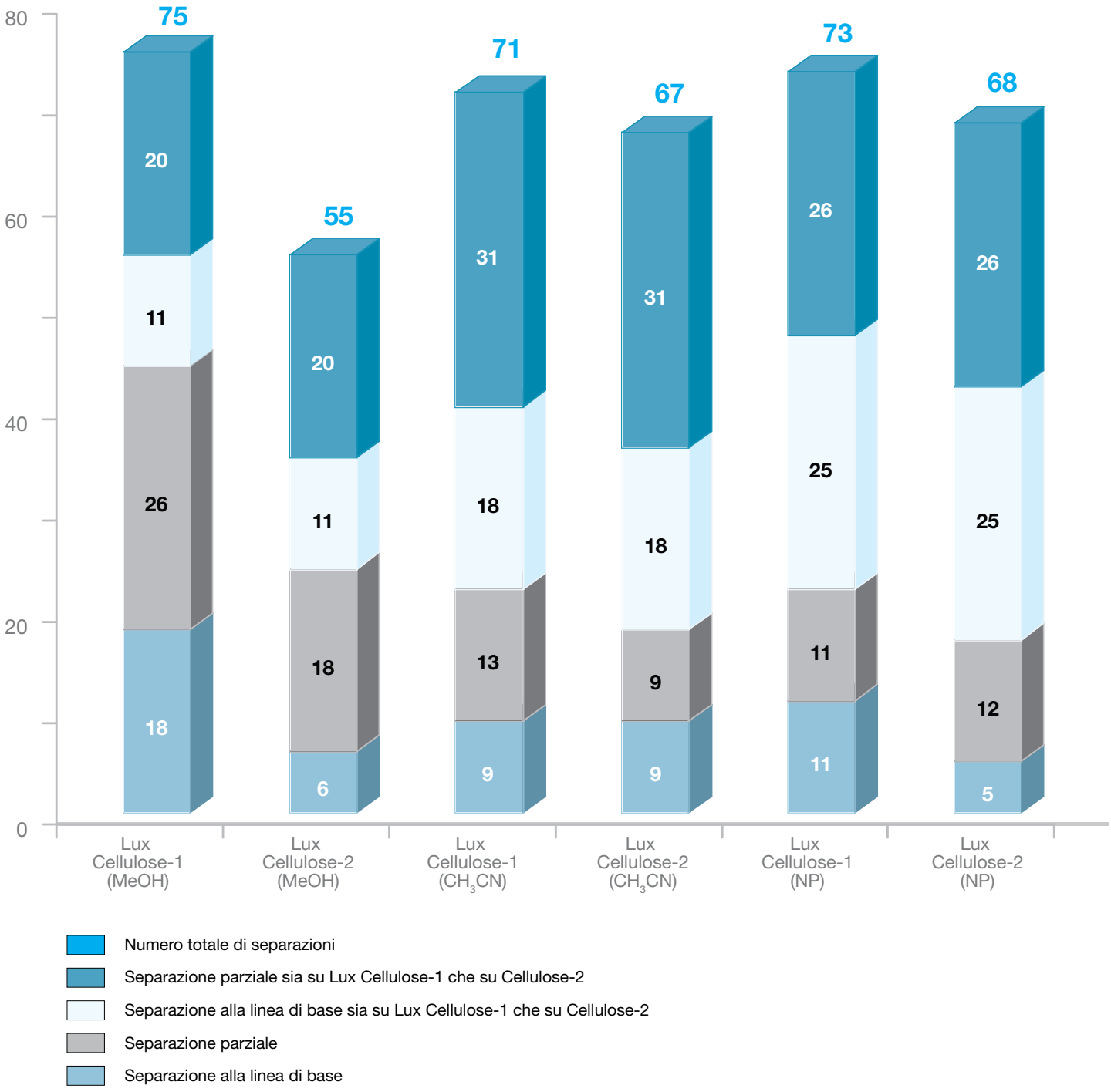
Column: Lux 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm ID
Part No.: 00G-4457-E0
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



Clenbuterol

Mobile Phase	Rs
Hexane:IPA	0.00
MeOH:IPA	0.00
CH_3CN	0.00

Figure 6.
Success Rates for over 180 Racemates on Lux™ Cellulose-1 and -2



Results and Discussion

Lux, a new line of polysaccharide-based chiral selective phases, has recently been introduced into the market. One phase, Lux™ Cellulose-1 is based on cellulose tris (3,5- dimethylphenyl carbamate) similar to other chiral phases on the market (e.g. CHIRACEL® OD-H®). The other phase, Lux Cellulose-2, is a new member to the family of polysaccharide based chiral selective phases and uses cellulose tris (3-chloro-4-methylphenyl carbamate) (**Figure 1**) as a chiral selector; this new chemistry delivers a unique selectivity versus other phases 2,3. In this study over 180 diverse compounds of pharmaceutical interest were screened on the Lux line of chiral selective phases as well as other comparative medias to better characterize the selectivity delivered by each Lux phase.

Table 1 summarizes the screening conditions used for each column; different types of mobile phases (NP, PO, and RP) as well as additives used (0.1 formic acid or acetic acid for acidic analytes or 0.1 % diethylamine for basic analytes).

Figures 2-5 show several representative examples of the different selectivities provided by Lux Cellulose-1 and Lux Cellulose-2 in chiral separations across normal phase, polar-organic and reversed phase separation modes. Representative compounds such as various ß-blockers, warfarin, sulconazole, milnacipran, and clenbuterol demonstrate the complementary behavior of Lux Cellulose-2 to the commonly used cellulose tris (3.5-dimethylphenylcarbamate) based CSPs (CHIRACEL® OD-H® and Lux Cellulose-1) in the separation of difficult racemates.

Figure 2 demonstrates the behavior of the two Lux phases in normal phase separations. While Lux Cellulose-1 generally demonstrates slightly better resolution and increased retention versus CHIRACEL® OD-H®, there are several cases where Lux Cellulose-2 is a better separation choice when using normal phase. While Bopindolol is equally well separated on the two Lux phases, Oxprenolol enantiomers are better resolved on Lux Cellulose-2. Toliprolol enantomers are separated on Lux Celluose-1 with spectacular resolution but at the expense of extensive retention for one of the enantomers. Lux Cellulose-2 barely separates racemic Oxprenolol under similar mobile phase conditions, but with minimal optimization (i.e. a reduction of IPA in the mobile phase) a better separation is achieved with Lux Cellulose-2 with significantly shorter analysis time. **Figure 3** shows additional normal phase enantomeric separations using Lux Cellulose-1, Lux Cellulose-2 and CHIRACEL® OD-H®. Such separations further demonstrate the complementary selectivity offered by Lux Cellulose-2 versus the cellulose tris (3,5-dimethylphenyl carbamate) phases Lux Cellulose-1 and CHIRACEL® OD-H®.

Figures 4 and **5** demonstrate the effect of mobile phase composition on chiral resolution. **Figure 4** demonstrates the dramatic changes in selectivity for each phase when traditional normal phase separation is substituted with polar organic separation mode using either acetonitrile or methanol as mobile phase. **Figure 5** shows changes in selectivity are observed in reversed phase mode. Different solvents can alter the steric structure of the polysaccharide backbone and the arrangement of binding sites[4], providing alternative selectivity for separating difficult chiral compounds. Such mobile phase alteration offers a powerful tool in developing and optimizing chiral separations.

Figure 6 compares the success rates of Lux Cellulose-1 and Lux Cellulose-2 in the analysis of over 180 racemates in normal phase or polar organic separation modes. The number of uniquely baseline resolved racemates are given at the bottom of the bar graph. The same selection criteria was applied to partially separated racemates. For example, Lux Cellulose-2 shows good chiral recognition in acetonitrile mobile phase with 9 baseline separations of racemates that could not be separated on Lux Cellulose-1. This complementary enantioselectivity of Lux Cellulose-2 over Lux Cellulose-1 is most evident in acetonitrile: IPA mobile phase mixtures, and is less pronounced in standard normal phase mixtures (hexane / IPA) and methanol mixtures.

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1. Y. Okamoto, Y. Kaida J. *Chromatography A* 666 (1994), 403-4192.
2. N. Matthijs, M. Maftouh, Y. Vander Heyden J. *Chromatography A* 1111 (**2006**), 48-613.
3. T. Huybrechts, G. Torrok, T. Vennekens, R. Sneyers, S. Vrielynck, I. Somers *LCGC Europe* June 1, **2007**
4. N. Maier, P. Franco, W. Lindner J. *Chromatography A* 906 (**2001**), 3-33

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Method Development for Reversed Phase Chiral LC/MS/MS Analysis of Stereoisomeric Pharmaceutical Compounds with Polysaccharide-based Stationary Phases

Philip J. Koerner, Kari Carlson, Liming Peng, Tivadar Farkas et al.
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Five different Lux® polysaccharide-based chiral stationary phases were explored in the reversed phase elution mode using mobile phases consisting of 0.1 % formic acid in acetonitrile or methanol to demonstrate the feasibility of LC/MS/MS analysis of a variety of acidic pharmaceutical racemates.

Introduction

Developing simple and straightforward reversed phase chiral LC separations coupled with highly sensitive MS detection is a challenging requirement for conducting drug metabolism and pharmacokinetic studies of stereoisomers. Polysaccharide derivatives are the most widely used chiral stationary phases (CSP) due to their wide chiral recognition and high loading capacity. As normal phase is favorable for their principal mechanism of chiral recognition – hydrogen bonding interaction – the majority of chiral separations with polysaccharide phases are performed in normal phase using hexane and alcohol modifiers as mobile phase components. However, these mobile phases are highly flammable and are not compatible with atmospheric pressure ionization (API) MS ion sources. The current research describes the effectiveness of an acidic mobile phase for the separation and detection of using acidic stereoisomers by ESI or APCI LC/MS/MS and for method development of these applications.

Experimental

Analytes: 15 acidic compounds of pharmaceutical interest were analyzed. The structures are shown in **Figure 2** and the MS ionization and mass reaction monitoring (MRM) transitions monitored are listed in **Table 1.**

Columns: Lux 3 µm Cellulose-1, 150 x 2.0 mm
Lux 3 µm Cellulose-2, 150 x 2.0 mm
Lux 3 µm Amylose-2, 150 x 2.0 mm
Lux 5 µm Cellulose-1, 250 x 4.6 mm
Lux 5 µm Cellulose-4, 250 x 4.6 mm
Lux 5 µm Cellulose-3, 250 x 4.6 mm
Lux 5 µm Amylose-2, 250 x 4.6 mm
Kinetex® 2.6 µm C18, 50 x 2.1 mm (used for achiral analysis)

Flow Rate: 0.2 mL/min (3 µm, 150 x 2.0 mm) or 1.0 mL/min (5 µm, 250 x 4.6 mm) – flow split to 0.25 mL/min into MS/MS

Temperature: 25 °C

Detection: UV @ 220 nm

Injection Volume: 5 µL (150 x 2.0 mm) or 10-20 µL (250 x 4.6 mm)

Mobile Phases: 1. 0.1 % Formic acid in Acetonitrile or Methanol
2. 5 mM Ammonium bicarbonate in Acetonitrile or Methanol (achiral analysis)
3. 5 mM Ammonium formate in Acetonitrile or Methanol (achiral analysis)
4. 5 mM Ammonium acetate in Acetonitrile or Methanol (achiral analysis)

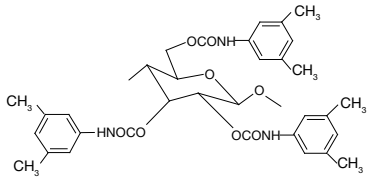
Instrument: HPLC System: Agilent® 1200 series equipped with binary pumpand autosampler (Agilent, Palo Alto, CA)MS
Detector: AB SCIEX™ 4000 LC/MS/MS Turbo V™ source with ESI or APCI probe
MS Detection: TurbolonSpray® – ESI or APCI in Positive or Negativelon Mode; MRM

Results and Discussion

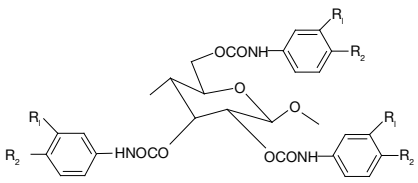
Chiral LC/MS/MS Experiments Five different polysaccharide-based chiral stationary phases (Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 **(Figure 1)** were explored in the reversed phase elution mode for the separation of a variety of acidic compounds of pharmaceutical interest in mobile phases consisting of 0.1% formic acid in acetonitrile or methanol and MS/MS detection.

Figure 1.
Structures of Polysaccharide-based Chiral Stationary (CSPs) Phases

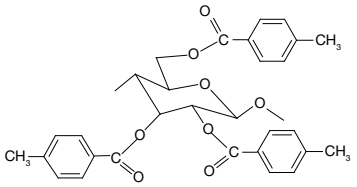
Lux Cellulose-1 Cellulose tris
(3,5-dimethylphenylcarbamate)



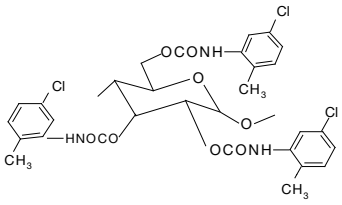
Lux Cellulose-2
Cellulose tris (3-chloro-4-methylphenylcarbamate)
with R_1=Cl and R_2=CH_3
or
Lux Cellulose-4
Cellulose tris (4-chloro-3-methylphenylcarbamate)
with R_1=CH_3 and R_2=Cl.



Lux Cellulose-3Cellulose tris-(4-methylbenzoate)



Lux Amylose-2 Amylose tris (5-chloro-2-methylphenylcarbamate)



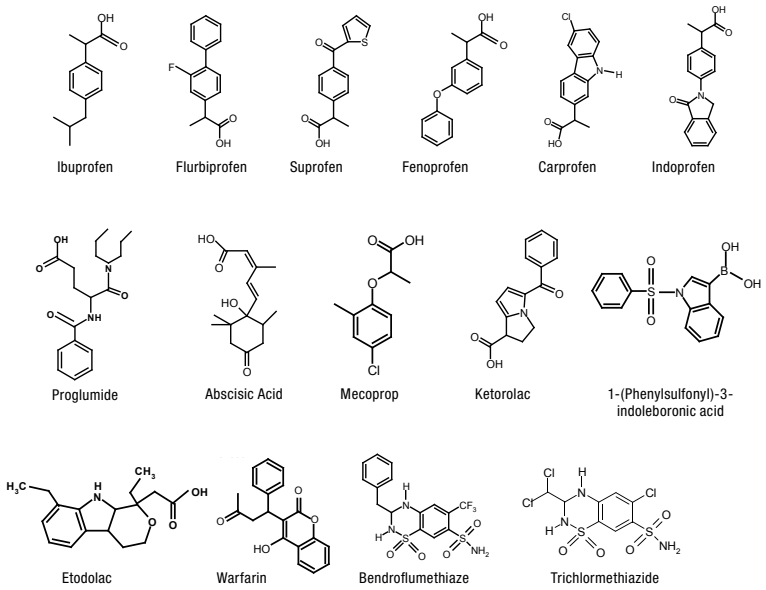
APPLICATIONS

Table 1.
MRM Transitions and Concentrations of Acidic Racemates

Compound	IS and MRM	Conc.*	Compounds	IS and MRM	Conc.*
Ibuprofen	ESI⁻205.1/160.1	100	Abscisic acid	APCI⁻263.0/152.7	50
Flurbiprofen	ESI⁻243.0/198.7	50	Mecoprop	ESI⁻214.1/141.7	50
Suprofen	ESI⁺261.1/111.0	50	Ketorolac	ESI⁻254.0/209.8 ESI⁺256.2/105.0	50
Fenoprofen	APCI⁻241.0/196.8	100	Etodolac	ESI⁻286.1/242.0	50
Carprofen	APCI⁻272.8/228.8	100	Warfarin	ESI⁻307.1/160.9 ESI⁺309.2/163.1	50
Indoprofen	ESI⁺282.1/236.1	50	Bendroflumethiaze	ESI⁻420.1/77.9	50
Proglumide	ESI⁻333.1/120.9	50	Trichlormethiazide	ESI⁻377.8/241.6	50
1-(Phenylsulfonyl)-3-indoleboronic acid	ESI⁻300.0/209.8	100			

* Conc. (ng/mL)

Figure 2.
Molecular Structures of Acidic Racemates



Selection of Mobile Phase Additives
As acidic analytes are present as anions in mobile phases of neutral pH their retention is not favorable on polysaccharide-based CSPs. Early elution and poor or no enantioseparation can result under these conditions. Acidic mobile phase additives are often required to suppress the dissociation of acidic analytes, resulting in increased retention and improved enantioselectivity.

Three volatile organic acids, (trifluoroacetic acid (TFA, pK_a 0.59), formic acid (FA, pK_a 3.75), and acetic acid (HAc, pK_a 4.76) were evaluated on Lux® Cellulose-1 and Lux Amylose-2 CSPs as acidic additives. In general, these additives provide similar enantioresolution for weakly acidic racemates (**Figures 3A-3C**) while the stronger acidic additive (TFA) performs better for the stronger acidic racemates (**Figures 3D–3F**). Formic acid provides comparable enantioseparations and peak shapes to TFA. Considering the "memory effect" of TFA commonly experienced on polysaccharide-based CSPs and its ion suppressing tendency in MS detection, formic acid was selected in preference over TFA in reversed phase mobile phase for the chiral separation and MS/MS detection of acidic racemates.

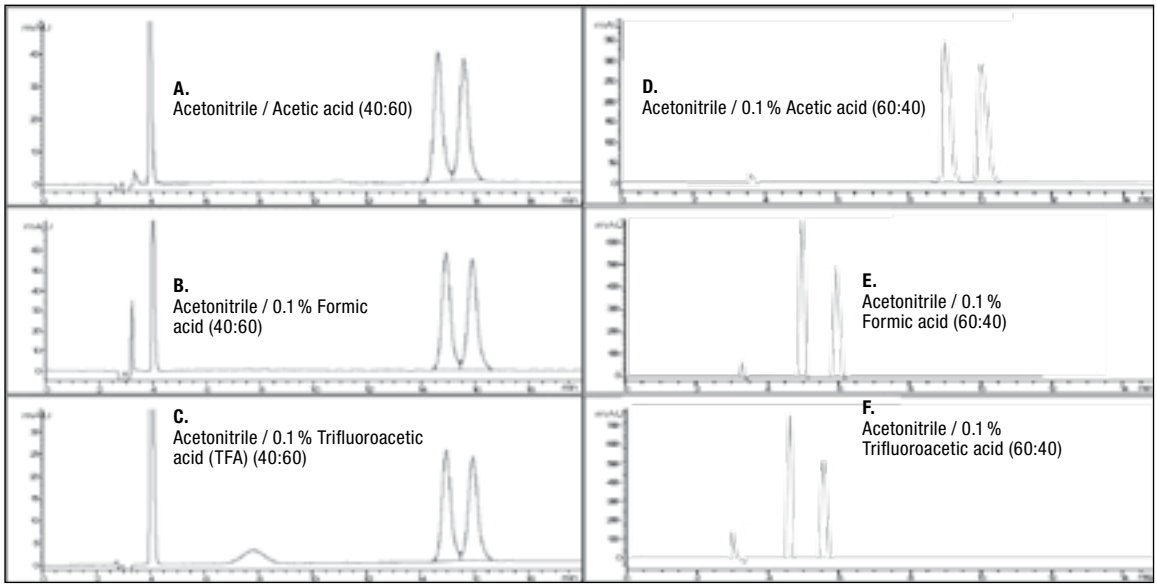
Effect of Mobile Phase Additives
The LC/MS/MS responses of acidic racemates with ESI negative (ESI-) mode in 5 mM ammonium formate, 5 mM ammonium acetate, and 5 mM ammonium bicarbonate containing mobile phases with either acetonitrile or methanol as organic modifier using an achiral column (Kinetex® 2.6 µm C18) were compared to responses in 0.1% formic acid **(Figures 4 and 5)**. The results show that MS/MS responses using 0.1% formic acid in acetonitrile or methanol are comparable to the responses using the other acid additives in ESI- mode, with the exception of carprofen which showed poor response with all of the acidic mobile phase additives. This shows that using 0.1% formic acid as acidic mobile phase additive is fully compatible with MS/MS detection and can be implemented as the first choice for mobile phase additive.

Effect of Organic Modifier on Chiral Resolution
Acetonitrile or methanol was the organic modifier used in chiral reversed phase HPLC. Decreasing the eluting strength of the mobile phase by decreasing the percentage of acetonitrile or methanol in the mobile phase will increase retention and resolution (**Figure 6**). However, once enantiomers elute later than about 10 minutes with only partial resolution, baseline separation can be rarely achieved by further decreasing the % organic modifier in the mobile phase. In our study, acetonitrile was more successful than methanol in providing chiral resolution on Lux CSPs in reversed phase (RP) mode (**Figure 8**), typically yielding sharper and narrower peaks.

Figure 3.
Effect of Acidic Additives on the Enantioseparation of Acidic Racemates in Reversed Phase

Bendroflumethiaze
Lux® 5 µm Cellulose-1
Part Number: 00G-4458-E0

Flurbiprofen
Lux 5 µm Amylose-2
Part Number: 00G-4471-E0



App ID 19326

App ID 19327

Column: As noted
Dimensions: 250 x 4.6 mm
Flow Rate: 1 mL/min
Temperature: 25 °C
Detection: UV @ 220 nm
Injection Volume: 10 µL
Mobile Phase: As noted

Figure 4.
LC/MS/MS Responses of Acidic Racemates in ESI- with Different Additives and Methanol

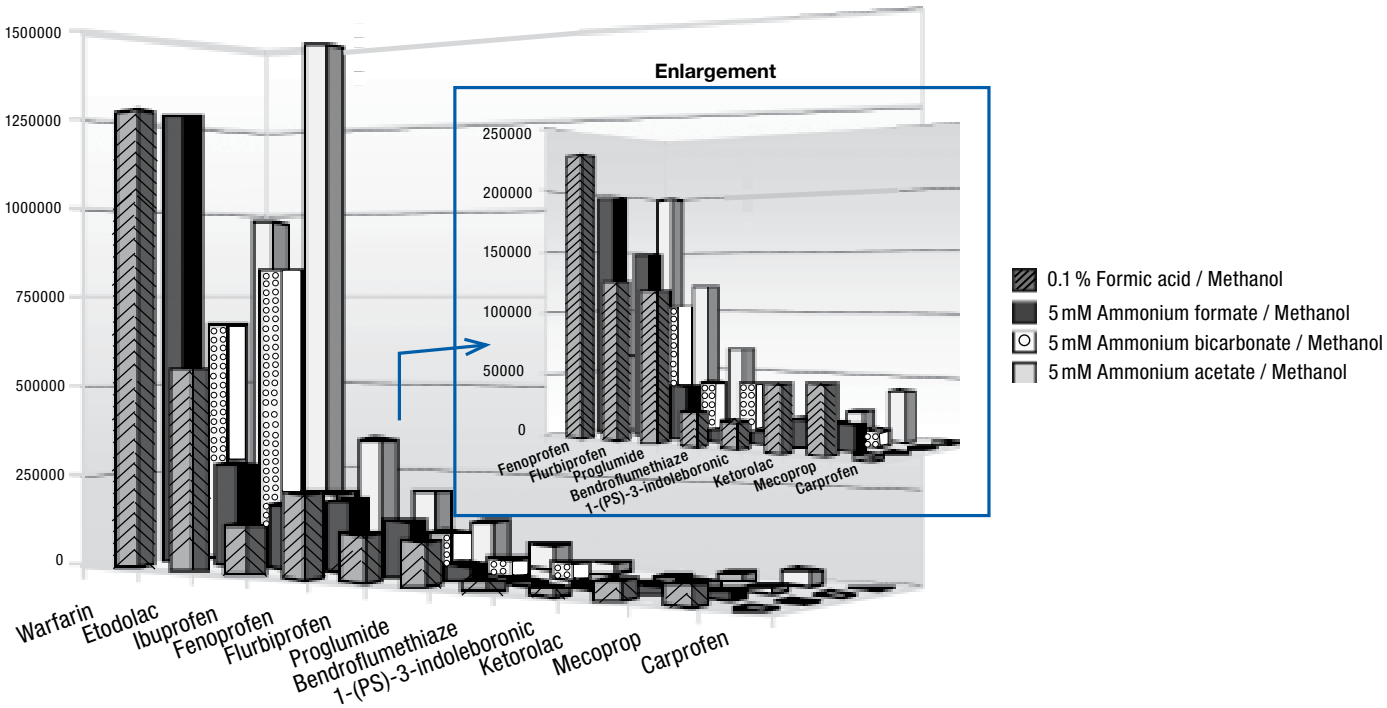


Figure 5.
LC/MS/MS Responses of Acidic Racemates in ESI- with Different Additives and Methanol

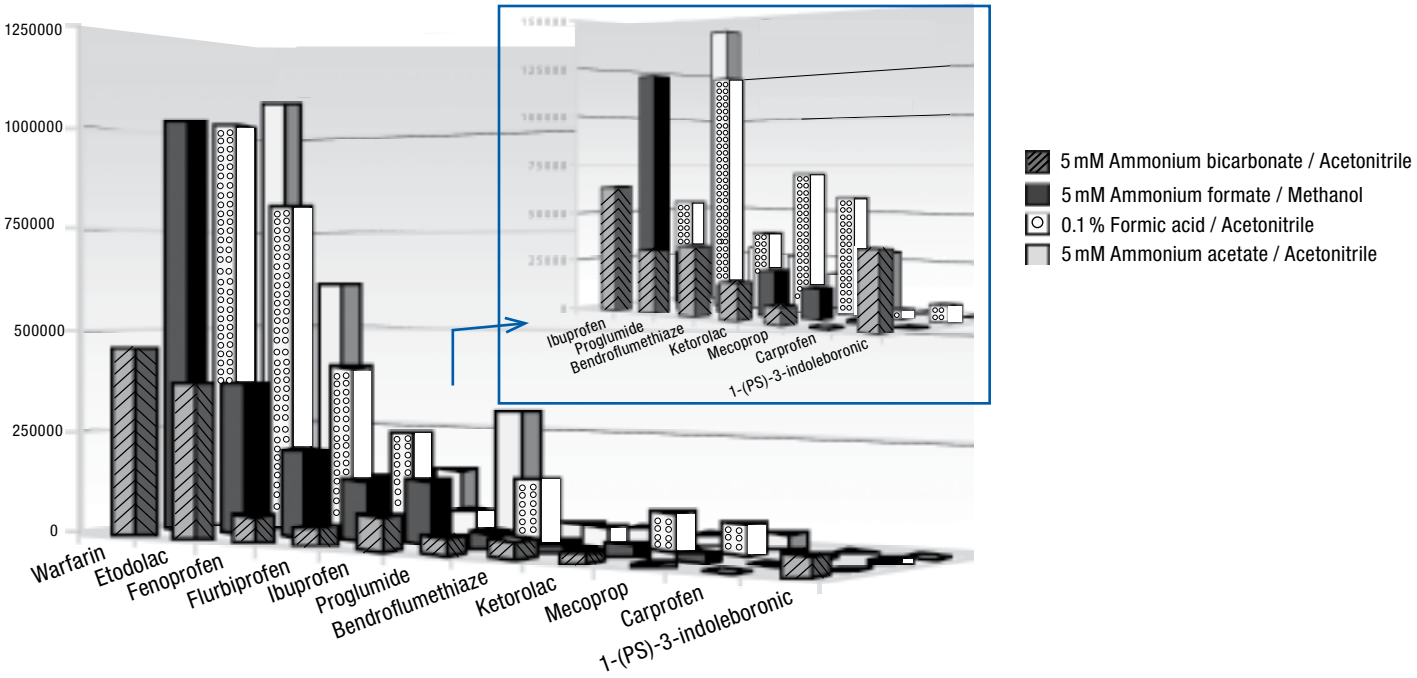


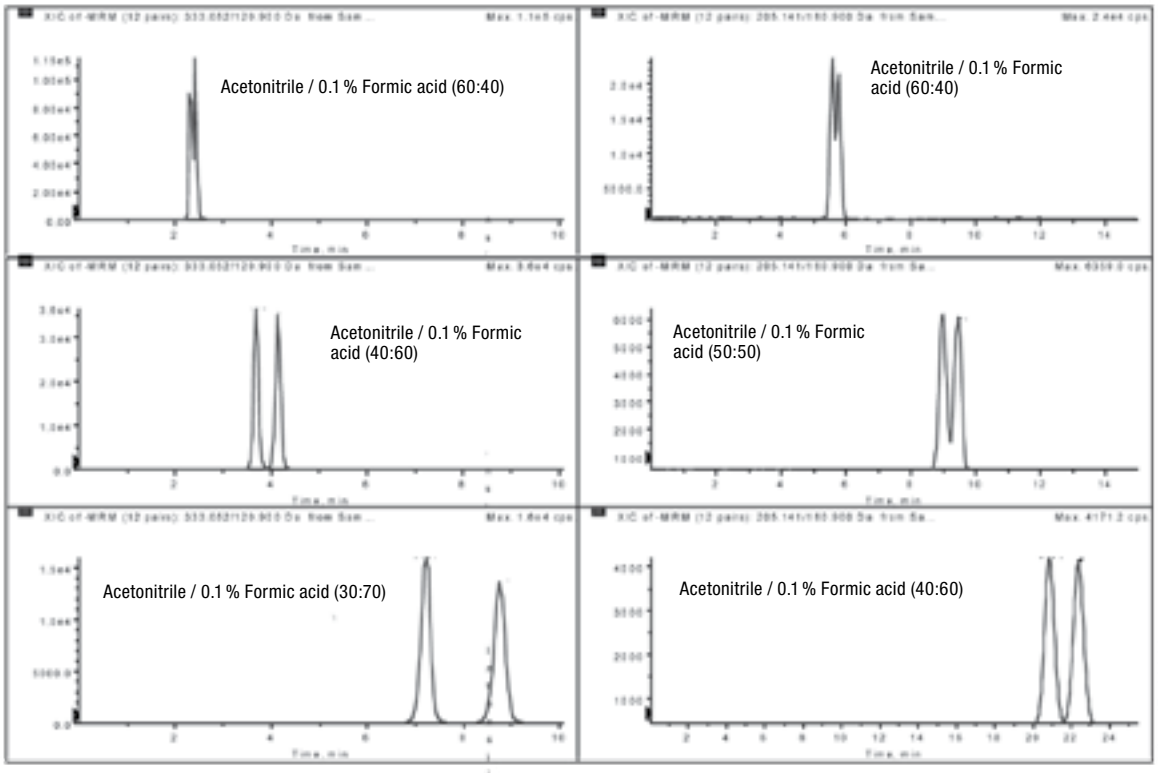
Figure 6.
Effect of Acidic Additives on the Enantioseparation of Acidic Racemates in Reversed Phase

Proglumide

Lux® 3 µm Cellulose-1
Flow Rate: 0.2 mL/min
Dimensions: 150 x 2.0 mm

Ibuprofen

Lux 5 µm Cellulose-3
Flow Rate: 1 mL/min
Dimensions: 250 x 4.6 mm



Column: As noted
Dimensions: As noted
Flow Rate: As noted
Temperature: 25 °C
Detection: Mass Spectrometer (MS)
Injection Volume: 5-20 µL
Mobile Phase: As noted
Sample: As noted

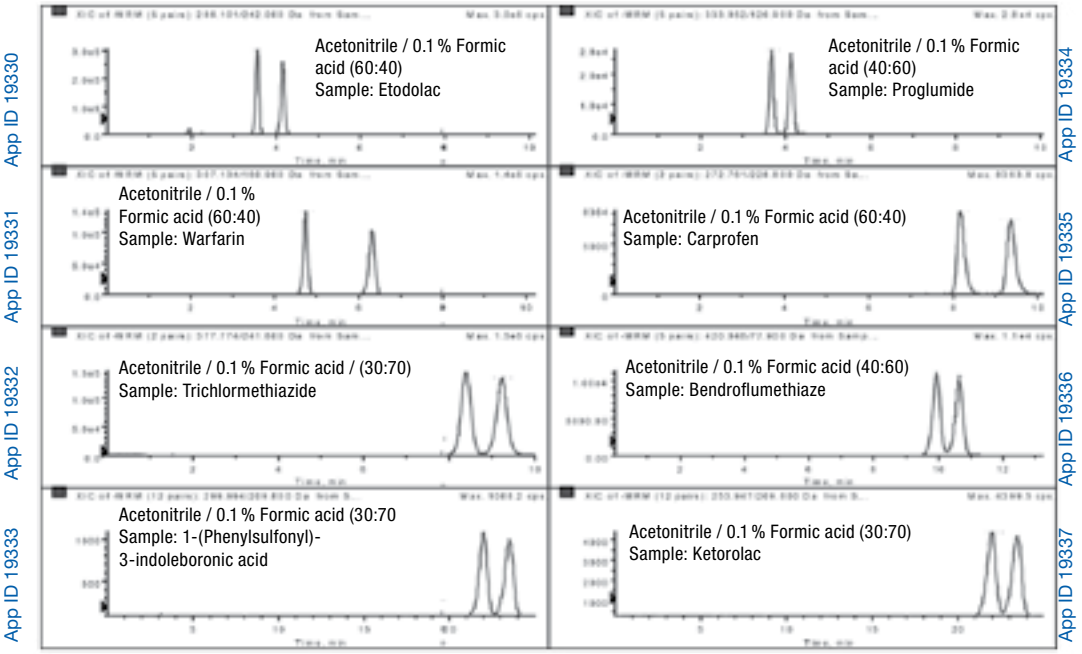
App ID 19328

App ID 19329

Chiral LC/MS/MS Applications

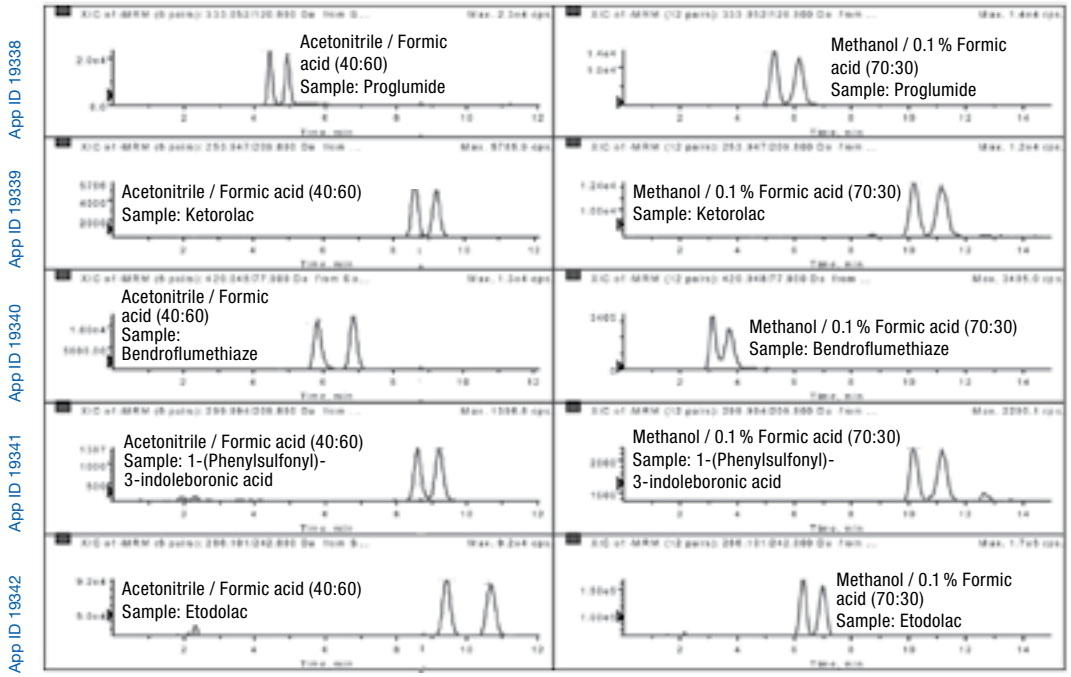
Figures 7-11 demonstrate 15 chiral separations on several different Lux chiral stationary phases. APCI negative (APCI-) mode was employed for the MS/MS detection of carprofen, abscisic acid, and fenoprofen as it provides much better MS/MS signals than ESI- for these acidic racemates. ESI positive (ESI+) mode was used for the MS/MS detection of suprofen and gave slightly improved signal intensity for warfarin and ketorolac versus ESI- (**Figure 10**). Most compounds evaluated here eluted in less than 10 min with baseline resolution in mobile phases of various elution strength. The results show that Lux® Cellulose-3 was most successful in separating acidic racemates (ten racemates), especially nonsteroidal anti-inflammatory drugs.

Figure 7.
Enantioseparations in Reversed Phase on Lux 3 µm Cellulose-1 with Acetonitrile



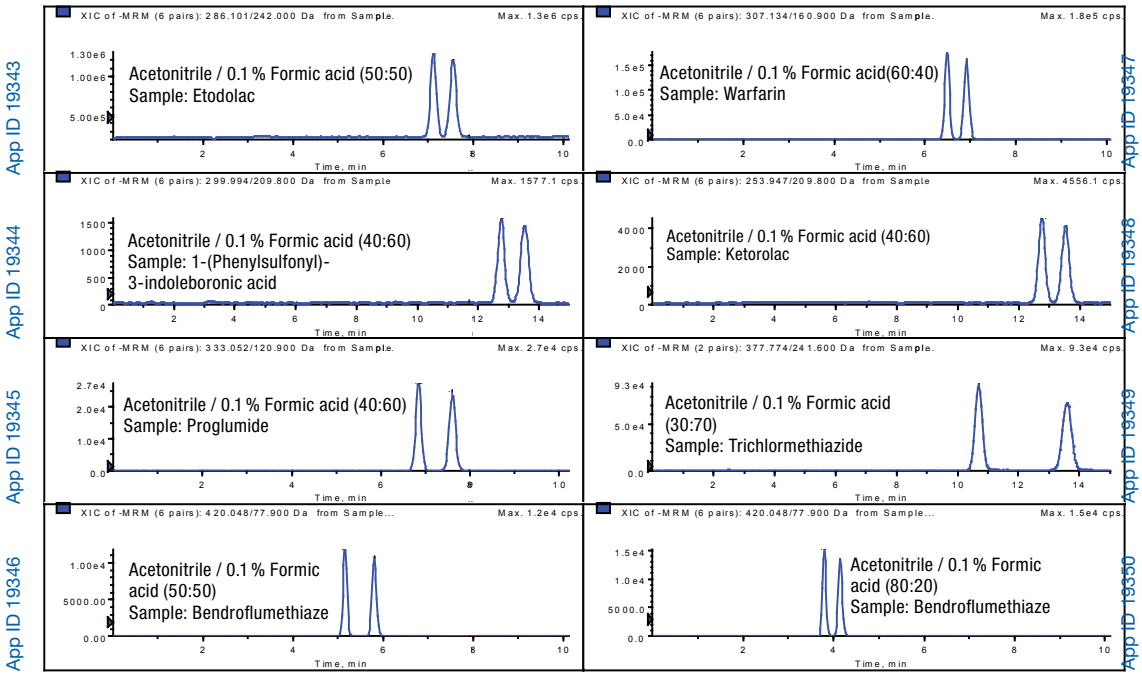
Column: Lux 3 µm Cellulose-1
Dimensions: 150 x 2.0 mm
Part No.: 00F-4458-B0
Flow Rate: 0.2 mL/min
Temperature: 25 °C
Detection: Mass Spectrometer (MS)
Injection Volume: 5 µL
Mobile Phase: As noted
Sample: As noted

Figure 8.
Enantioseparations on Lux 3 µm Cellulose-2 with Acetonitrile or Methanol as Modifier



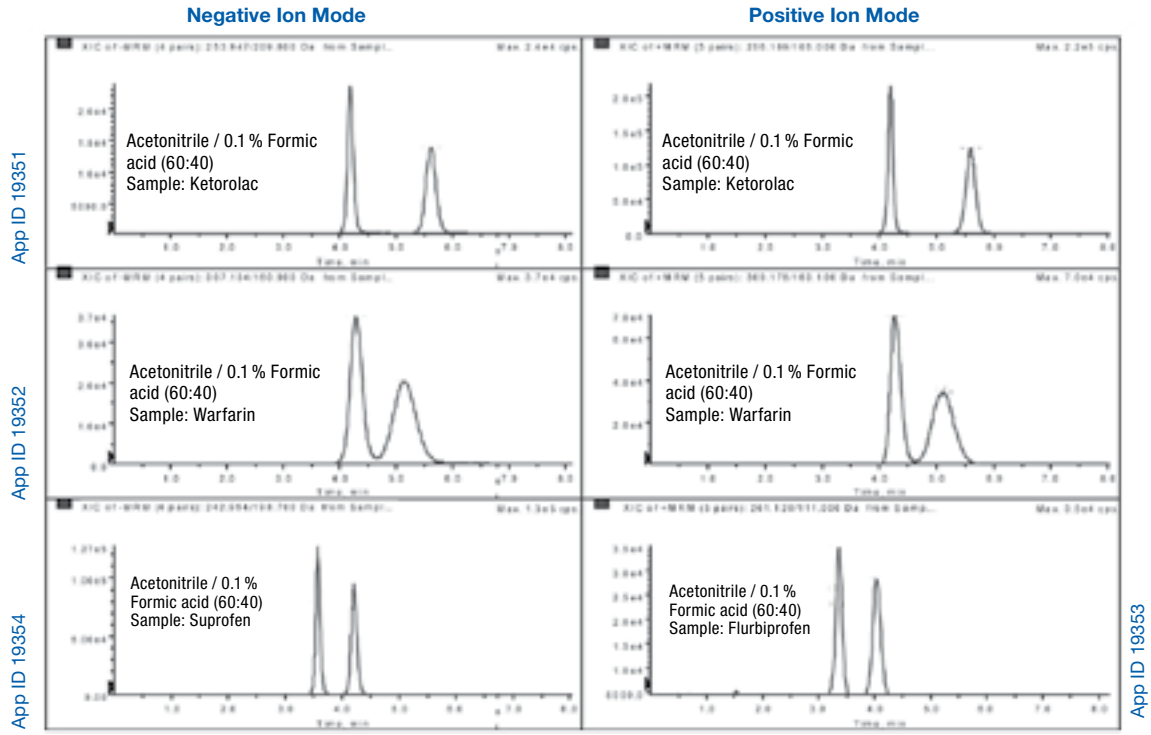
Column: Lux 3 µm Cellulose-2
Dimensions: 150 x 2.0 mm
Part No.: 00F-4456-B0
Flow Rate: 0.2 mL/min
Temperature: 25 °C
Detection: Mass Spectrometer (MS)
Injection Volume: 5 µL
Mobile Phase: As noted
Sample: As noted

Figure 9.
Enantioseparations on Lux® 5 µm Cellulose-4 with Acetonitrile



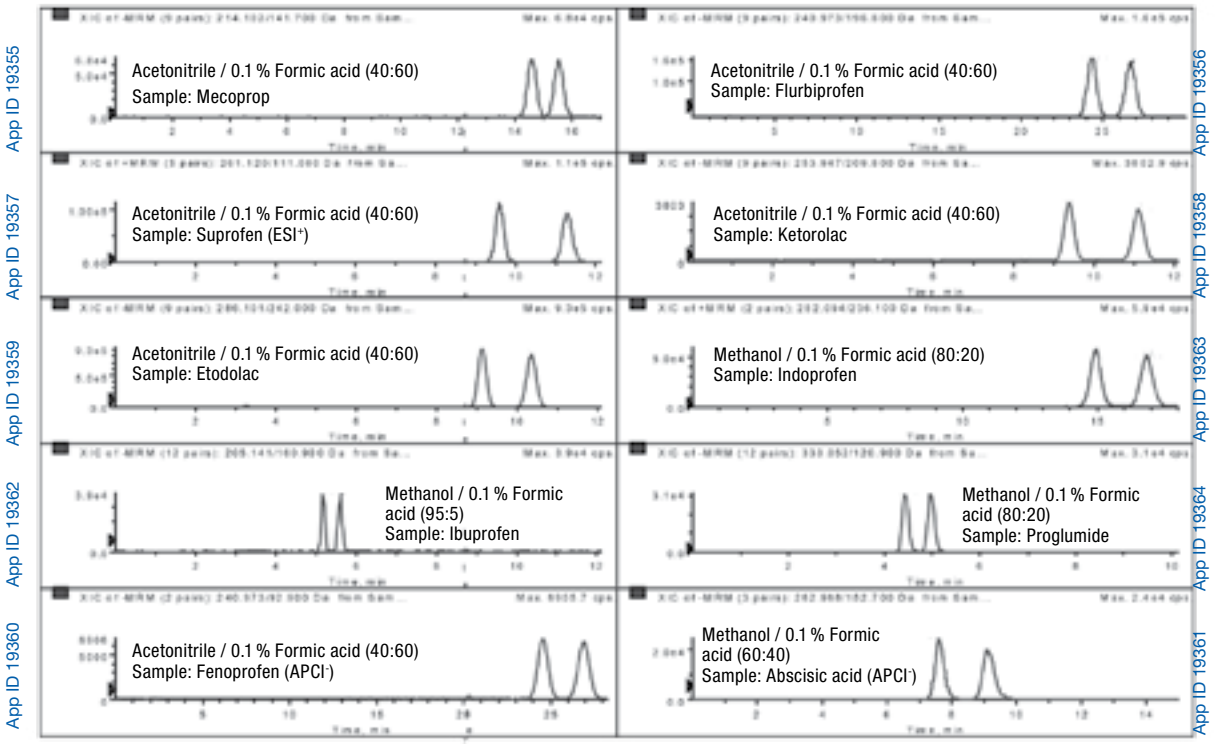
Column: Lux 5 µm Cellulose-4
Dimensions: 250 x 4.6 mm
Part No.: 00G-4491-E0
Flow Rate: 1 mL/min
Temperature: 25 °C
Detection: Mass Spectrometer (MS)
Injection Volume: 20 µL
Mobile Phase: As noted
Sample: As noted

Figure 10.
Enantioseparations in Reversed Phase on Lux 3 µm Amylose-2 with Acetonitrile



Column: Lux 3 µm Amylose-2
Dimensions: 150 x 2.0 mm
Part No.: 00F-4471-B0
Flow Rate: 0.2 mL/min
Temperature: 25 °C
Detection: Mass Spectrometer (MS)
Injection Volume: 5 µL
Mobile Phase: As noted
Sample: As noted

Figure 11.
Enantioseparations in Reversed Phase on Lux® 5 µm Cellulose-3 with Acetonitrile or Methanol as Modifier



Column: Lux 5 µm Cellulose-3
Dimensions: 250 x 4.6 mm
Part No.: 00G-4493-E0
Flow Rate: 1 mL/min
Temperature: 25 °C
Detection: Mass Spectrometer (MS)
Injection Volume: 20 µL
Mobile Phase: As noted
Sample: As noted

Conclusions

The chiral LC/MS/MS analysis of fifteen different acidic racemates are successfully demonstrated on the polysaccharide-based CSPs Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 in reversed phase (RP) elution mode

Formic acid is a good first choice for an acidic RP mobile phase additive as it leads to increased retention and improved enantioselectivity for acidic enantiomers and is also compatible with MS/MS detection.

Increasing the percentage of organic modifier (acetonitrile or methanol) in the RP mobile phase has the expected effect of decreasing retention and enantioselectivity. Adjusting the organic modifier content of the mobile phase is therefore essential to optimizing chiral resolution.

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Performance Evaluation of Immobilized and Coated Polysaccharide Chiral HPLC Columns Using Generic Screening Mobile Phase Systems

Zdravko Milanov, Liming Peng, Jeff Layne, Marc Jacob et al.
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Two chiral stationary phases (Lux® Cellulose-1 and CHIRALPAK®IB) that consist of the same chiral selector, cellulose tris(3,5-dimethylphenylcarbamate), were evaluated. The major difference between the two phases is that the first phase (Lux Cellulose-1) is prepared by coating the underlying silica with the modified polysaccharide, while the second phase is an immobilized phase in which the polysaccharide is covalently bonded to the underlying silica. This covalent linkage allows for the use of an extended range of solvents (e.g., THF, DMF, acetone, ethyl acetate, methylene chloride) that are not compatible with coated chiral stationary phases (CSPs). In this study, we sought to determine if this expanded solvent range increased the success rate of chiral separations using the immobilized CSP.

Introduction

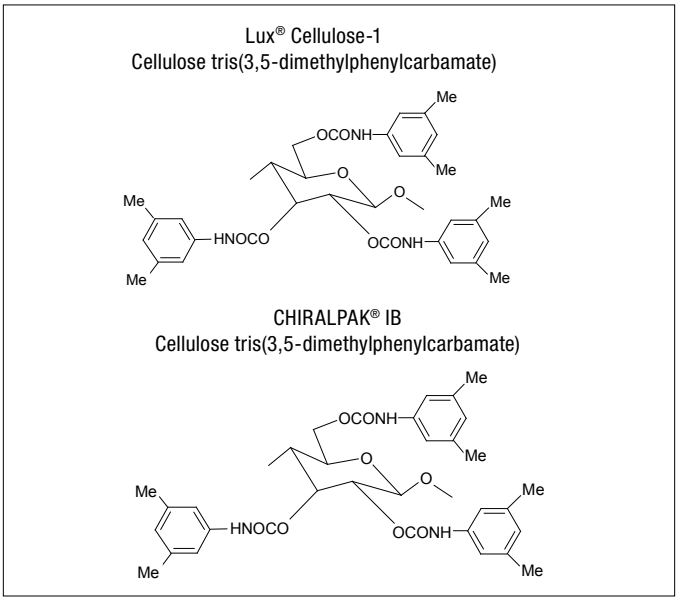
Modified polysaccharide-based stationary phases are the most widely used CSPs due to their broad-spectrum chiral selectivity and high loading capacity. Most separations performed using polysaccharide CSPs are performed in normal phase using solvents such as hexane and alcohol, and these conditions have been proven to be very favorable for chiral recognition mechanisms.

The majority of polysaccharide-based CSPs are coated phases, in which the stationary phase is not covalently bonded to the underlying silica. Recently, immobilized polysaccharide CSPs, in which the polysaccharides are covalently linked to the silica, have also become available. Immobilized CSPs allow for the use of more aggressive solvents, such as chlorinated solvents (e.g., methylene chloride) or ethyl acetate, which cannot be used with conventional coated phases due to solubility issues.

There has been speculation that the expanded solvent range of immobilized CSPs might increase the selectivity options and, hence, lead to enhanced enantiorecognition relative to coated CSPs. However, there is a lack of extensive comparative studies to determine if this is indeed the case. Thus, in this study, we have sought to determine if an immobilized CSP (CHIRALPAK IB) exhibits significantly increased chiral separation success rates as compared to a coated CSP (Lux Cellulose-1), when evaluated under generic normal phase screening conditions (including the use of chlorinated solvents). The two chiral stationary phases evaluated in this study consist of the same chiral selector, cellulose tris(3,5-dimethylphenylcarbamate) as depicted in **Figure 1**.

Generic screens in Normal Phase (NP) are common in the industry, as NP is favorable for the principal mechanisms of chiral recognition. The majority of chiral separations with polysaccharide phases are performed using hexane and alcohol modifiers. Previous work has identified the different selectivities offered between Isopropyl alcohol (IPA) and Ethanol (EtOH), and we have used these solvents in our generic screen of both columns. Chlorinated solvents and ethyl acetate may also be used as NP modifiers to offer a different analyte solvent selectivity; however, these cannot be used on coated polysaccharide columns. Our generic screen incorporated the addition of the above solvents only for the immobilized phase.

Figure 1.
Structures of cellulose based chiral stationary phase used in this study



Material and Methods

All analyses were performed using an HPLC Agilent® 1100 series (Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler and a quaternary pump. Chiral chromatographic separations followed by UV detection were performed using Lux Cellulose-1 (coated phase) and CHIRALPAK® IB (immobilized phase) HPLC columns with dimensions 250 x 4.6 mm ID packed with 5 µm particles. The system flow rate was set to 1 mL/min and the column temperature was ambient unless noted otherwise Mobile Phase Conditions used for each column are described in **Table 1.**

Table 1.
Mobile phase conditions used in this study.

CSP	Mobile Phase (MP)
Coated Phase Lux® Cellulose-1	80:20:0.1 Hex:IPA:DEA; and 85:15:0.1 Hex:EtOH:DEA
Immobilized Phase CHIRALPAK® IB	80:20:0.1 Hex:IPA:DEA; 85:15:0.1 Hex:EtOH:DEA; 65:35:0.1 Hex:EtOAc:Ethanolamine; and 65:35:0.1 Hex:$CHCl_3$:Ethanolamine

DEA = Diethyl amine; IPA = Isopropyl alcohol; $CHCl_3$ = Chloroform; Hex = Hexane; EtOH = Ethanol; EtOAc = Ethyl acetate

APPLICATIONS

Results and Discussion

To evaluate the enantioresolution between coated and immobilized CSP, 51 chemical compounds of pharmaceutical interest were analyzed under various mobile phase conditions. In the first set of experiments, IPA was used as a modifier with DEA as additive. Out of those 51 compounds, 22 were resolved on either CSPs using 80:20:0.1 Hexane:IPA:DEA as mobile phase. **Table 2** summarizes the difference in enantioselectivity using those conditions.

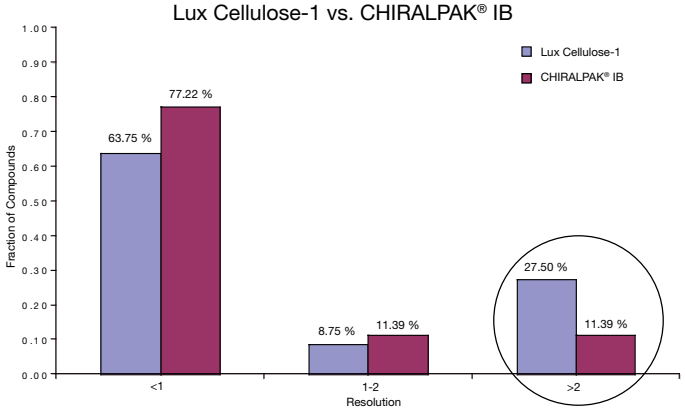
Table 2.
Comparison of enantioresolution of 22 racemates between coated and immobilized CSP using 80:70:0.1 Hex:IPA:DEA as organic modifier

Compounds	Lux® Cellulose-1	CHIRALPAK® IB	Compounds	Lux Cellulose-1	CHIRALPAK® IB
Tetrahydrozoline	√	x	Toliprolol	√	√
Metoprolol	√	x	Bisoprolol	√	Partial
Tetramisole	√	x	Sulfconazole	x	Partial
Halofantrine	√	√	Orphenadrine	√	√
Bopindolol	√	Partial	Mianserin	√	x
Bupranolol	√	Partial	1,1-Dihydroxy-6,6-Dimethylbiphenyl	√	√
Carazolol	Partial	x	Methoxy-p-tolyl sulfoxide	√	x
Metomidate	√	x	Prilocaine	√	Partial
Mephenesin	√	x	Nifedpine	x	√
Oxazapam	√	√	Bupivacaine	√	x
Oxprenolol	x	√	Disopyramide	Partial	x

	Lux Cellulose-1	CHIRALPAK® IB
√ Baseline Resolution $R_s > 1.5$	17	7
x No resolution $R_s < 0.8$	3	10
Partial Resolution $0.8 < R_s < 1.5$	2	5

The percentage of compounds that showed a resolution > 2 on coated Lux Cellulose-1 is over twice that observed on immobilized CHIRALPAK® IB using Hexane/IPA as mobile phase as represented in **Figure 2**.

Figure 2.
Percentage of compounds showing resolution > 2 using IPA as modifier.



Flow Rate: 1.0 mL/min
Temperature: 25 ˚C
Detection: 220 nm
Mass Loaded: 10 µg

The same 51 compounds were analyzed under different mobile phase conditions using EtOH as organic modifier with DEA as additive. Out of those 51 compounds, 28 analytes were resolved on either CSP using 85:15:0.1 Hexane:EtOH:DEA as mobile phase. **Table 3** summarizes the difference in enantioselectivity using those conditions.

Table 3.
Comparison of enantioresolution of 28 racemates between coated and Immobilized CSP using 85:15:0.1 Hex EtOH:DEA as organic modifier

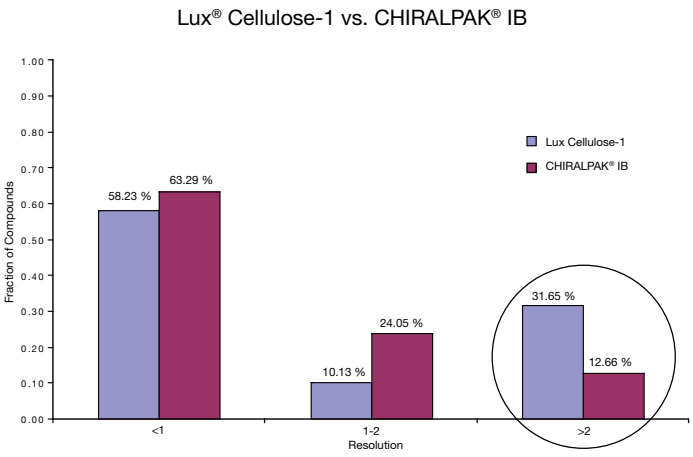
Compounds	Lux® Cellulose-1	CHIRALPAK® IB	Compounds	Lux Cellulose-1	CHIRALPAK® IB
DL-B-Hydroxyphenethylamine	x	Partial	Carazolol	√	Partial
Miconazole	√	Partial	Toliprolol	√	√
Tetrahydrozoline	√	x	Bisoprolol	√	Partial
Metoprolol	x	√	Sulfconazole	√	√
Acebutolol	Partial	x	Orphenadrine	√	Partial
Tetramisole	√	√	Mianserin	√	Partial
Halofantrine	√	√	1,1-Dihydroxy-6,6-Dimethylbiphenyl	Partial	√
Bopindolol	√	√	Methoxy-p-tolyl sulfoxide	√	x
Bupranolol	√	x	5-Methyl-5-phenyl-hydantoin	Partial	x
Metomidate	√	Partial	Nifedpine	x	√
Mephenesin	√	Partial	Bupivacaine	Partial	x
Oxazapam	√	√	Omeprazole	√	√
Oxprenolol	√	√	Indapamide	x	√
Prilocaine	Partial	Partial	Bendroflumethiazide	x	√

	Lux Cellulose-1	CHIRALPAK® IB
√ Baseline Resolution $R_s > 1.5$	18	13
x No resolution $R_s < 0.8$	5	6
Partial Resolution $0.8 < R_s < 1.5$	5	9

APPLICATIONS

The percentage of compounds that showed a resolution > 2 on coated Lux® Cellulose-1 is over twice that observed on immobilized CHIRALPAK IB using Hexane/EtOH as mobile phase as represented in **Figure 3**.

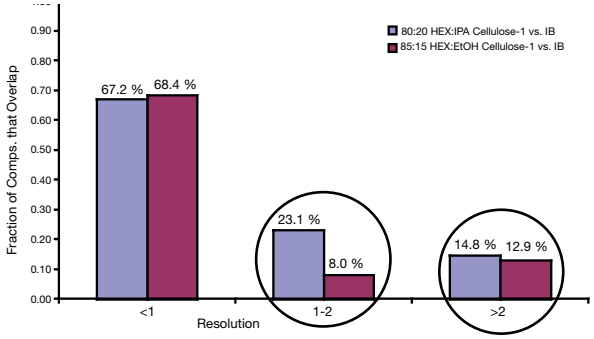
Figure 3.
Percentage of compounds showing resolution > 2 using EtOH as modifier



Flow Rate: 1.0 mL/min
Temperature: 25 °C
Detection: 220 nm
Mass Loaded: 10 µg

Interestingly, although both columns shared similar chiral selector, cellulose tris(3,5-dimethylphenylcarbamate), there was very little correlation between the compounds that were resolved on the coated phase and those that were resolved on the immobilized phase (< 25 % of the racemates were resolved on both columns under identical running conditions) as depicted in **Figure 4**.

Figure 4.
Extent of Complementary Selectivity between Coated and Immobilized CSPs in Hex:IPA and Hex:EtOH



% Match of Compounds Resolved on both Lux Cellulose-1 and CHIRALPAK IB for Different Resolution Ranges

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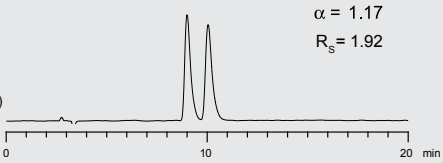
APPLICATIONS

A few representative examples of the effect on the mobile phase composition on the chiral separation on coated and immobilized CSP are presented on **Figure 5.** The data clearly indicates that under the same mobile phase conditions, coated CSP Lux Cellulose-1 has a greater separation properties over immobilized CSP.

Figure 5.
Effect of Organic Modifier on Lux Cellulose-1 (coated) and CHIRALPAK® IB (immobilized) CSPs

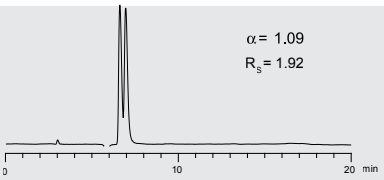
Mephenesin

Columns: Lux® 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



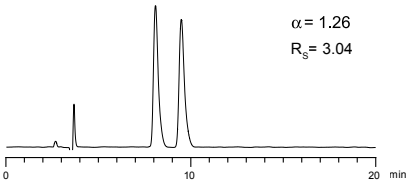
App ID 19195

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



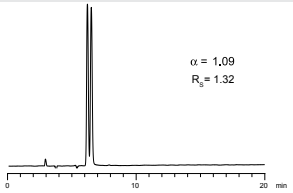
App ID 19196

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19197

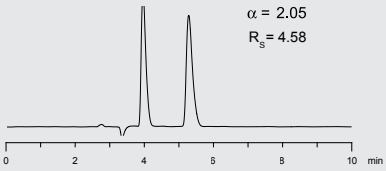
Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19198

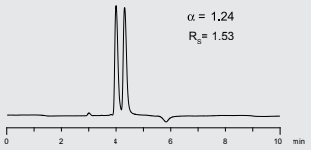
Orphenedrine®

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



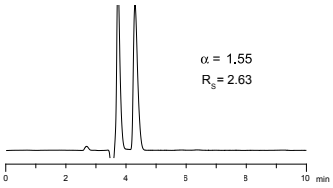
App ID 19199

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



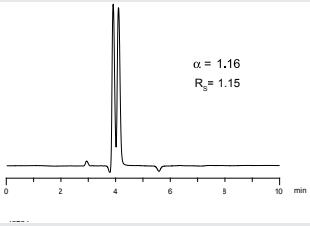
App ID 19200

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19201

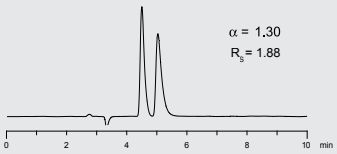
Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19202

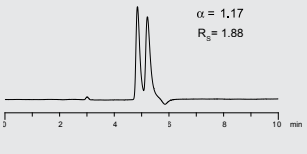
Prilocaine

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



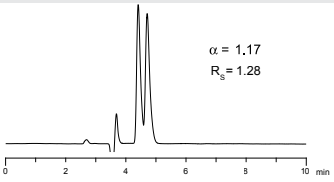
App ID 19203

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



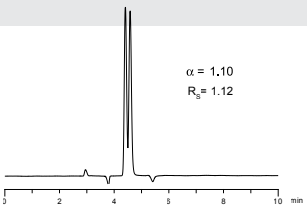
App ID 19204

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19205

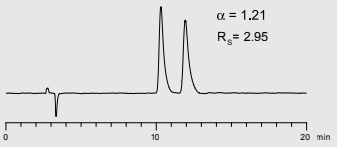
Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19206

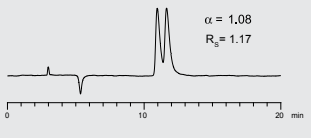
Tetramisole

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Heptane/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



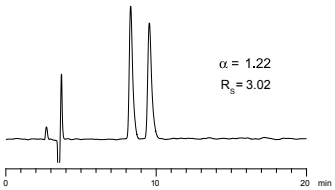
App ID 19207

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Heptane/IPA/DEA (80:20:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



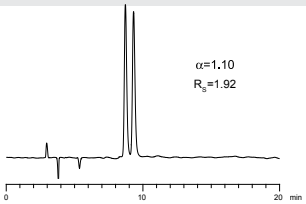
App ID 19208

Columns: Lux 5 µm Cellulose-1
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm



App ID 19209

Columns: CHIRALPAK® 5 µm IB
Dimensions: 250 x 4.6 mm
Mobile Phase: Hex/EtOH/DEA (85:15:0.1)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm

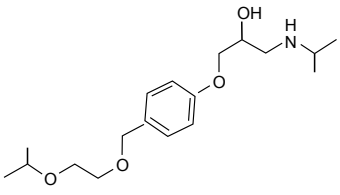


App ID 19210

APPLICATIONS

Immobilized CSP can be used with mobile phases of various natures, ranging from the so called “standard solvents “ such as acetonitrile, alcohols, and alkanes recommended for coated CSPs to mobile phase containing “non-standard” solvents such as chlorinated solvents, ethyl acetate, tetrahydrofuran (THF) and methyl tertiary butyl ether (MTBE). **Figures 6-8** show separation of three chiral analytes using “standard” and “non-standard” solvents indicating that using chloroform ($CHCl_3$) or ethyl acetate (EtOAc) in the mobile phase does not necessarily improve the chiral separation.

Figure 6.
Bisoprolol on CHIRALPAK® IB



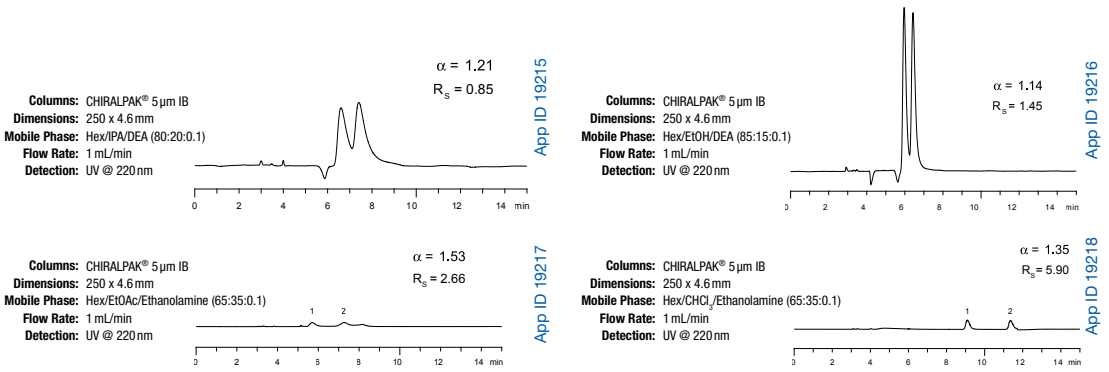
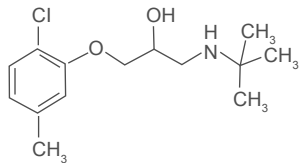


Figure 7.
Bupranolol on CHIRALPAK® IB



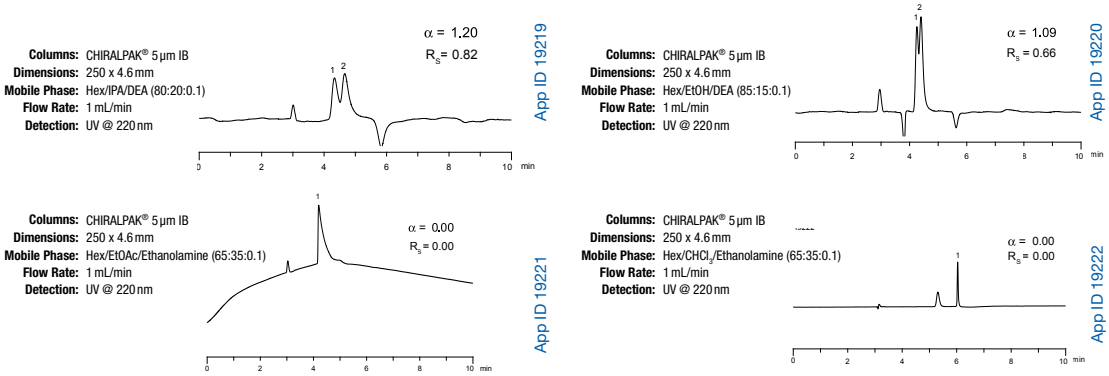
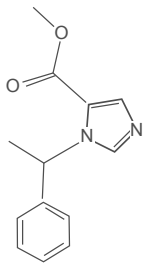
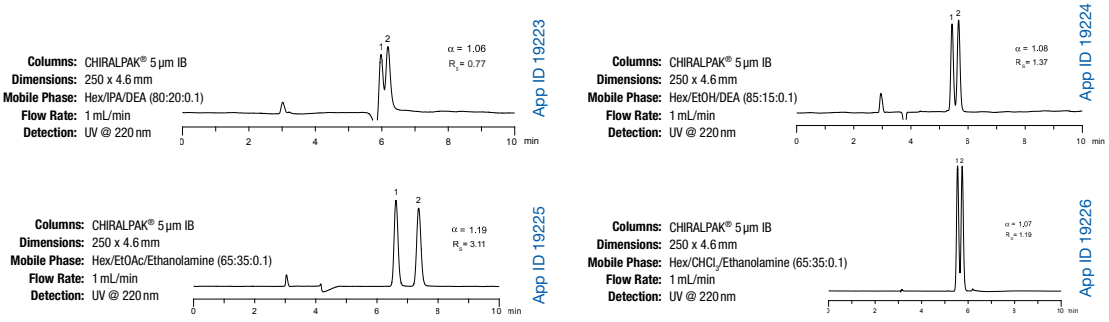


Figure 8.
Metomidate on CHIRALPAK® IB





APPLICATIONS

Of the 51 unique compounds investigated using various NP solvent systems only 18 were partially or fully resolved using Chiralpak IB as summarized in **Table 4**. The option of using non-standard solvents on immobilized CSPs clearly did not offer any advantages over coated CSP for those 51 compounds.

Table 4.

Compounds	80:20:0.1 Hex:IPA:DEA	85:15:0.1 Hex:EtOH:DEA	65:35:0.1 Hex:EtOAc:Ethanolamine	65:35:0.1 Hex:$CHCl_3$:Ethanolamine
1,1-Dihydroxy-6,6-Dimethylbiphenyl	√	√	x	Partial
Bisoprolol	Partial	Partial	√	√
Bopindolol	Partial	√	x	√
Bupranolol	Partial	x	x	√
Disopyramide	x	x	√	x
Mephenesin	Partial	Partial	√	x
Methoxy-p-tolyl sulfoxide	x	x	Partial	x
Metomidate	x	Partial	√	Partial
Metoprolol	x	√	√	x
Mianserin	x	Partial	Partial	Partial
Miconazole	x	Partial	Partial	x
Orphenadrine	√	Partial	√	√
Oxprenolol	√	√	√	√
Prilocaine	Partial	Partial	√	√
Sulfconazole	√	√	√	√
Tetramisole	Partial	√	Partial	x
Toliprolol	√	√	√	x
Zopiclone	x	x	√	√

√ Baseline resolution: $R_s > 1.5$
x No resolution $R_s < 0.8$
Partial resolution $0.8 < R_s < 1.5$

Conclusions

In this Technical Note, we compared the chiral separation success rate of an immobilized CSP (CHIRALPAK IB) to a conventional coated CSP (Lux® Cellulose-1) under generic normal phase screening conditions using 51 different racemates of pharmaceutical interest.

Using the conventional mobile phase of Hexane/IPA/DEA, the coated Lux Cellulose-1 column was able to resolve 17 racemates, while the immobilized CHIRALPAK® IB column was only able to resolve 7 racemates. Using another conventional mobile phase (Hexane/Ethanol/DEA), the coated phase column was able to resolve 18 racemates, while the immobilized phase column was only able to resolve 13 racemates with baseline or greater resolution.

The overall resolution success rate of the coated Lux Cellulose-1 column was 45 % (Rs > 2) using two mobile phases (Hexane/IPA/DEA and Hexane/EtOH/DEA) compared to the overall success rate of 37 % for the immobilized CHIRALPAK IB columns using four different mobile phases (Hexane/IPA/DEA, Hexane/EtOH/DEA, Hexane/Chloroform/Ethanolamine, and Hexane/Ethyl acetate/Ethanolamine).

Overall, the data indicates that, under generic normal phase screening conditions, traditional coated CSPs display greater enantioselectivity in terms of % of compounds resolved with greater than baseline resolution than do immobilized CSPs.

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HPLC Enantioseparation of N-FMOC α-Amino Acids Using Lux® Polysaccharide-Based Chiral Stationary Phases Under Reversed Phase Conditions

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Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

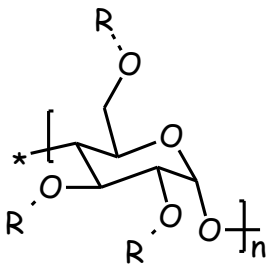
In this technical note, we report the chiral separation of the most common 19 FMOC protected α-amino acids derivatives under reversed phase separation mode using Lux polysaccharide-based chiral stationary phases. All FMOC α-amino acids analyzed in this study are baseline resolved with an analysis time below 25 min in isocratic conditions. The order of elution as well as the enantiomer identification are also reported.

Introduction

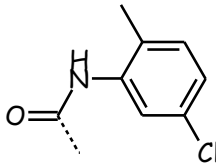
N-Fluorenylmethoxycarbonyl (FMOC) α-amino acids are important building blocks for the solid phase synthesis of peptides.[1] After the development of FMOC/tBu strategy[2] for solid phase peptide syntheses, FMOC α-amino acids have become the raw materials of choice for the preparation of synthetic peptides. Using this methodology, long peptides (up to 100 residues) can be prepared in a few days with high yield from micro molar (g) up to molar scale (kg). As the number of amino acids residues increases, the final purity and overall yield of the peptide produced is directly affected by the chemical and chiral purity of the protected amino acids used. Currently, for the most common commercially available FMOC protected α-amino acids (19 natural amino acids), the expected enantiomeric purity is > 99.0 % enantiomeric excess (ee) for the L form and sometimes the purity required must be >= 99.8 % ee. This level of precision can only be achieved by very few analytical techniques, chiral HPLC being one of them. The main advantages of chiral HPLC analysis over other techniques are speed, detection level, and ease of use. HPLC is also used on a regular basis by the peptide chemists to analyze purified fractions as well as peptide purity. In this application, we report for the first time, the chiral separation of the most common commercially available FMOC protected α-amino acids under reversed phase conditions using polysaccharide-based chiral stationary phases (CSPs) depicted in **Figure 1**.[3]

Figure 1.
Structures of Polysaccharide-Based CSPs

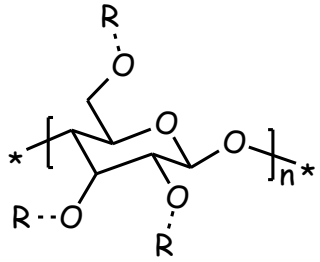
Amylose Backbone



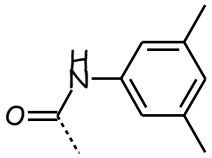
Lux Amylose-2 (Amy-2)



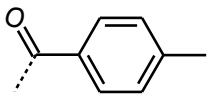
Cellulose Backbone



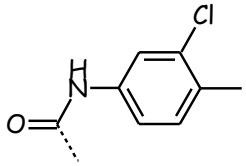
Lux Cellulose-1 (Cell-1)



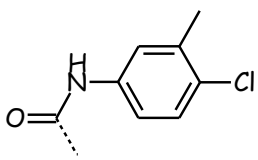
Lux Cellulose-3 (Cell-3)



Lux Cellulose-2 (Cell-2)



Lux Cellulose-4 (Cell-4)



Materials 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. FMOC protected L and D amino acids used in this study were provided by Bachem® (Bubendorf, Switzerland). All solvents were purchased from EMD (San Diego, CA, USA).

HPLC Conditions:

Columns:	Lux 5 µm Cellulose-1	250 x 4.6 mm	00G-4458-E0
	Lux 5 µm Cellulose-2	250 x 4.6 mm	00G-4456-E0
	Lux 5 µm Cellulose-3	250 x 4.6 mm	00G-4492-E0
	Lux 5 µm Cellulose-4	250 x 4.6 mm	00G-4490-E0
	Lux 5 µm Amylose-2	250 x 4.6 mm	00G-4471-E0
Flow Rate:	1 mL/min		
Temperature:	Ambient		
Detection:	UV @ 220 nm		
Injection Volume:	5 µL		
Sample concentration:	2 mg/mL in Methanol (MeOH) or Acetonitrile (ACN) (pure FMOC amino acids enantiomer L and D were mixed in a ratio of 2:1 (L:D))		

Results and Discussion

Five different polysaccharide-based chiral stationary phases (CSPs) Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 (**Figure 1**) were explored in the reversed phase (RP) HPLC enantioseparation of the 19 most common FMOC protected a-amino acids.

Due to the acidic nature of FMOC amino acid derivatives and based on our previous extensive screening work in RP mode,[4] it was decided to use trifluoroacetic acid (TFA) or formic acid (FA) as acidic additives with acetonitrile (ACN) or methanol (MeOH) as organic modifier (see experimental conditions). Those mobile phases are arguably the most used in RP mode. All the analysis were performed in isocratic mode with run time below 25 min.

Initial screening of the Lux CSPs was performed with 0.1 % TFA/ACN in a volume ratio of 40:60. For retention time (Rt) < 6 min and resolution (Rs) < 1.5 (no baseline resolution), the amount of ACN was decreased in order to improve retention and chiral recognition. If no chiral separation was obtained with ACN as modifier, columns were screened with 0.1 % FA/MeOH in a volume ratio of 20:80. In general, we observed more retention with TFA as an additive than with FA when using ACN as modifier and as expected ACN elution power is stronger than MeOH. Quite a few FMOC amino acids can be separated with either ACN or MeOH as modifier.

Table 1 summarizes all the separations and chiral recognition observed after performing RP screening using the protocol described above. As shown in **Table 1**, all the amino acids tested were successfully resolved on at least one of the five Lux polysaccharide-based CSPs. In the case of Ile, Leu, Met, Phe, and Val FMOC derivatives, baseline resolution was achieved on the five CSPs.

Table 1.
Chiral Recognition of the 19 Most Common FMOC Protected α-Amino Acids

Baseline resolution | Chiral separation | No resolution

FMOC-AA-OH	Cell-1	Cell-2	Cell-3	Cell-4	Amy-2
FMOC-Ala-OH					
FMOC-Arg(Pbf)-OH					
FMOC-Asn(Trt)-OH					
FMOC-Asp(OtBu)-OH					
FMOC-Cys-(Trt)-OH					
FMOC-Gln(Trt)-OH					
FMOC-Glu(OtBu)-OH					
FMOC-His(Trt)-OH					
FMOC-Ile-OH					
FMOC-Leu-OH					
FMOC-Lys-(Boc)-OH					
FMOC-Met-OH					
FMOC-Phe-OH					
FMOC-Pro-OH					
FMOC-Ser(tBu)-OH					
FMOC-Thr(tBu)-OH					
FMOC-Trp(Boc)-OH					
FMOC-Tyr(tBu)-OH					
FMOC-Val-OH					

Under our RP screening protocol, Cellulose-2 was the most successful phase with 18 chiral recognitions followed by Cellulose-3 as represented in **Figure 2**.

Figure 2.
Enantioselectivity Comparison Between Polysaccharide-Based CSPs

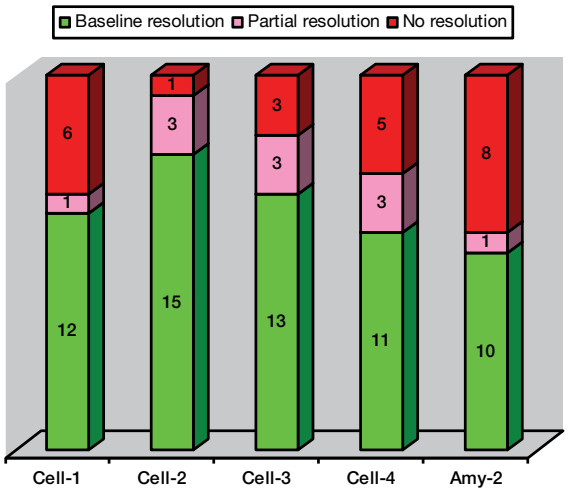


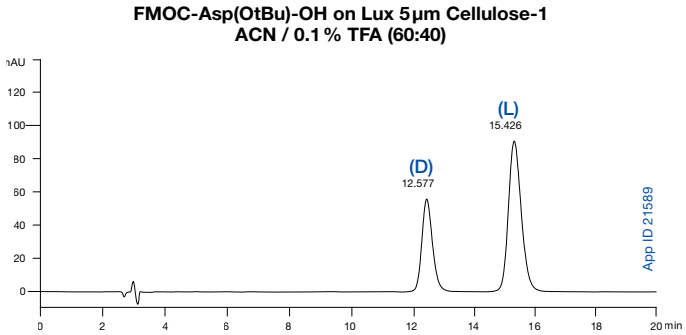
Table 2 describes some of the best separation observed for each FMOC amino acid screened. Retention time for both enantiomers, alpha value, resolution achieved, and order of elution are provided. All the separation reported are baseline resolved and the run time is less than 25 min. Interestingly, Trityl (Trt) side chain protected FMOC amino acids such as His, Asn, and Cys derivatives are more challenging to separate and baseline resolution is only achieved using Cellulose-2, Cellulose-3, and Cellulose-1, respectively. Selected chiral separation of FMOC-Asp(OtBu)-OH and FMOC-Tyr(tBu)-OH are shown in **Figure 3**.

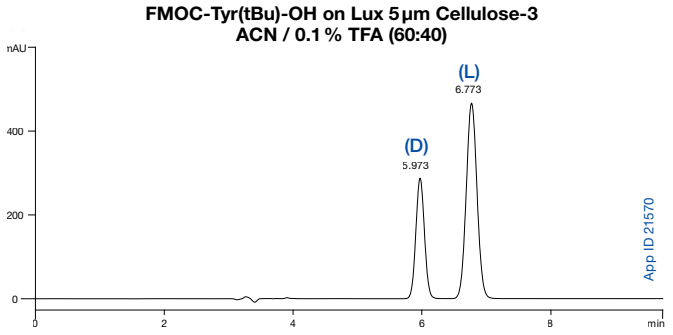
Table 2.
Optimal RP HPLC Enantioseparation of the 19 Most Common FMOC Protected α-Amino Acids

FMOC-AA-OH	CSP	Mobile Phase	Rt_1[a]	Rt_2[a]	Alpha	Rs	App ID[b]
FMOC-Ala-OH	Cell-3	MeOH / 0.1 % TFA (80:20)	7.165	9.551	1.55	5.63	21550
FMOC-Arg(Pbf)-OH	Cell-1	ACN / 0.1 % TFA (70:30)	8.547	9.991	1.24	2.71	21580
FMOC-Asn(Trt)-OH	Cell-2	ACN / 0.1 % TFA (55:45)	20.825	23.124	1.10	1.60	21873
FMOC-Asp(OtBu)-OH	Cell-1	ACN / 0.1 % TFA (60:40)	12.577	15.426	1.28	4.18	21589
FMOC-Cys-(Trt)-OH	Cell-4	MeOH / 0.1 % TFA (90:10)	9.969	11.375	1.20	1.79	21641
FMOC-Gln(Trt)-OH	Cell-4	ACN / 0.1 % TFA (70:30)	7.184	8.866	1.39	4.47	21631
FMOC-Glu(OtBu)-OH	Cell-1	ACN / 0.1 % TFA (60:40)	13.979	16.652	1.23	3.55	21590
FMOC-His(Trt)-OH	Cell-1	ACN/ 0.1 % FA (60:40)	4.865	5.783	1.39	2.33	21582
FMOC-Ile-OH	Cell-3	ACN / 0.1 % TFA (40:60)	12.22	13.64	1.15	2.86	21553
FMOC-Leu-OH	Cell-3	MeOH / 0.1 % TFA (90:10)	4.56	5.654	1.64	3.60	21647
FMOC-Lys-(Boc)-OH	Cell-3	ACN / 0.1 % TFA (50:50)	5.615	6.52	1.33	3.59	21546
FMOC-Met-OH	Cell-1	ACN / 0.1 % TFA (60:40)	11.423	13.064	1.18	2.96	21559
FMOC-Phe-OH	Cell-1	ACN / 0.1 % TFA (60:40)	18.965	21.963	1.18	2.80	21585
FMOC-Pro-OH	Cell-4	ACN / 0.1 % TFA (60:40)	5.865	6.818	1.32	3.31	21643
FMOC-Ser(tBu)-OH	Cell-3	ACN / 0.1 % TFA (40:60)	8.654	9.599	1.16	2.87	21549
FMOC-Thr(tBu)-OH	Cell-4	ACN / 0.1 % TFA (60:40)	7.69	8.92	1.26	3.78	21629
FMOC-Trp(Boc)-OH	Cell-1	ACN / 0.1 % TFA (80:20)	8.179	9.576	1.25	3.28	21586
FMOC-Tyr(tBu)-OH	Cell-3	ACN / 0.1 % TFA (60:40)	5.973	6.773	1.26	2.89	21570
FMOC-Val-OH	Cell-1	ACN / 0.1 % TFA (60:40)	11.669	15.052	1.37	3.90	21579

[a] Highlighted in blue is the retention time for the D enantiomer
[b] To view the full application enter the App ID into the search field on our website at www.phenomenex.com/ChiralAppSearch

Figure 3.
RP HPLC Enantioseparations of FMOC-Asp(OtBu)-OH and FMOC-Tyr(tBu)-OH





Conclusion

Five different polysaccharide-based chiral stationary phases were explored in reversed phase HPLC for the separation of the 19 most common FMOC protected α-amino acids. Under our RP screening protocol, Lux® Cellulose-2 was the most successful phase with 18 chiral recognitions (15 baseline resolved) followed by Lux Cellulose-3.

All FMOC amino acids evaluated were fully resolved (Rs>1.5) in less than 25 min analysis time by RP separation mode. TFA as acidic additive and Acetonitrile as organic modifier are the best choice combination for successful chiral separation of FMOC α-amino acids derivatives.

Based on this study, we feel confident that with a proper screening protocol most of the FMOC protected amino acids can be resolved with the five polysaccharide-based chiral stationary phase used in this study.

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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]
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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	Isothipend yl	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
Car vedilol	Mianserine	*Suprofen*
Clorphenamine	Nadolol	Terbutaline
Chlorthalidone	*Naringenin*	Terta tolol
Dimethindene	Nicardipine	Tetramisol
Ephedrine	Nimodipine	V era pamil
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

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

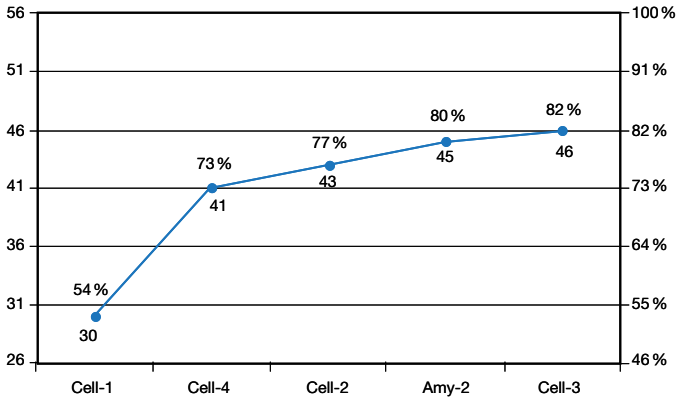
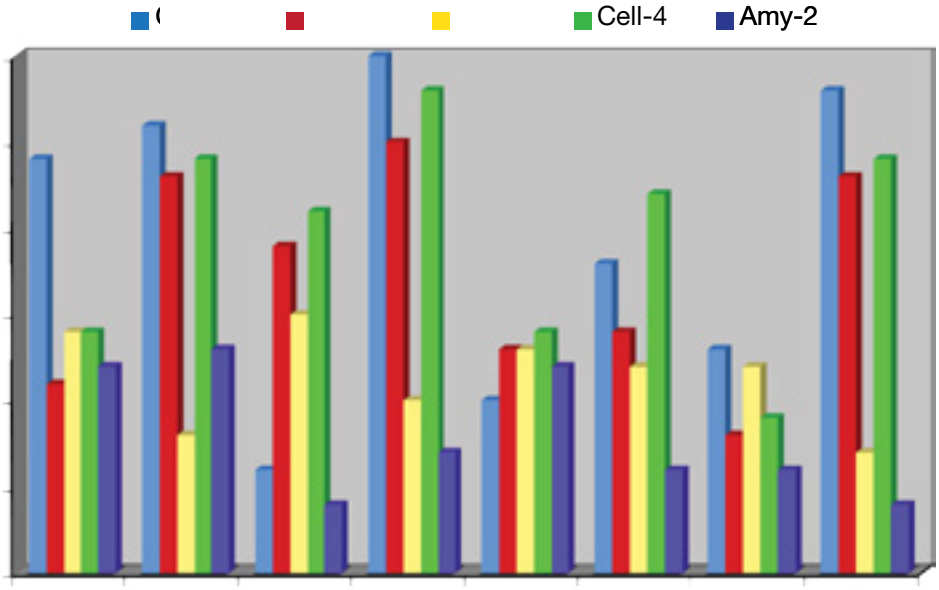
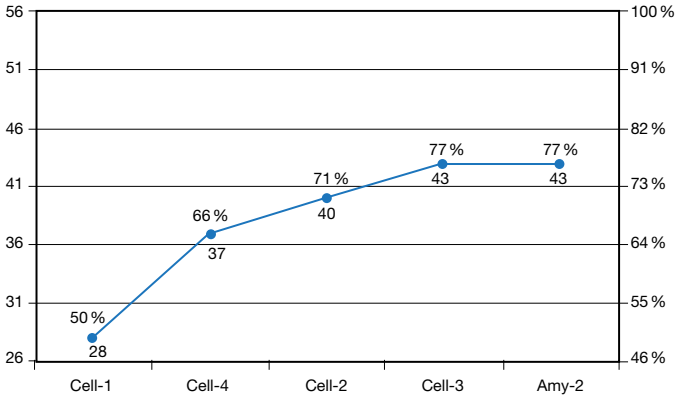


Figure 1.
Number of baseline separations for mobile phase A through H.



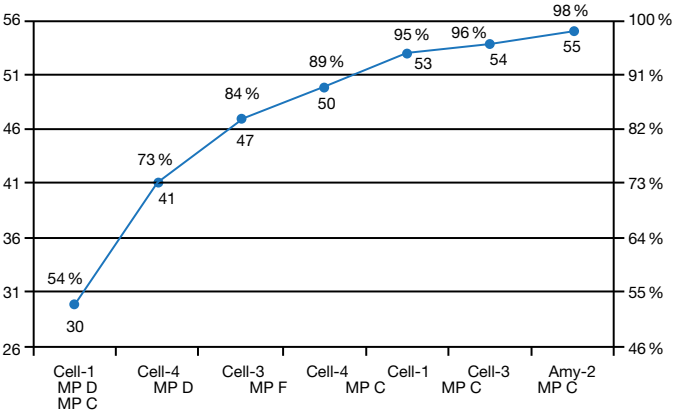
The cumulative baseline separations of the five Lux polysaccharide-based chiral columns using a mobile phase of CO_2/(2PrOH with 0.1 % IPA and 0.1 % TFA), 80/20, v/v (H) are shown in **Figure 3**. In the same way as before, 43 of the 56 pharmaceutical compounds are baseline resolved. In fact, only four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



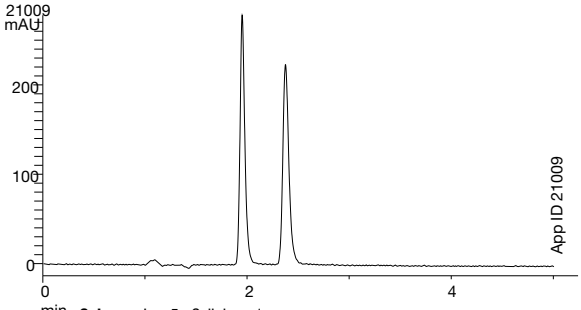
By using 7 chromatographic systems, which require three mobile phases (C, D, and F) and four Lux CSPs (Cellulose-1, Cellulose-3, Cellulose-4, and Amylose-2), 55 of the 56 test group compounds are baseline separated (Figure 4).

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



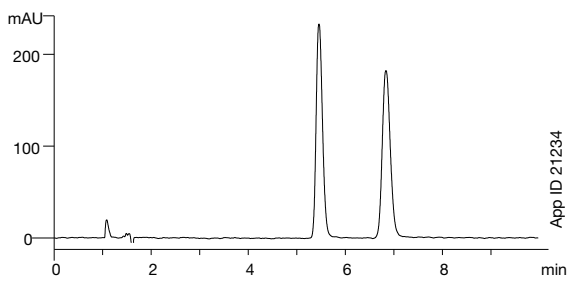
In **Figures 5a** and **5b**, some typical SFC separations are represented. All chiral separations with resolutions greater than 1.5 can be found on our application search web page www.phenomenex. com/Application/Search.

Figure 5a.
Chiral separation of Alprenolol by SFC



Column: Lux 5u Cellulose-1
Dimensions: 250 x 4.6mm
Mobile Phase: CO2/(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 4.6mm
Mobile Phase: CO2/(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.

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5. De Klerck K.; Mangelings D.; Vander Heyden Y. *J. of Supercritical Fluids* **2013**, 80, 50-59.

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Purification Techniques

Purification of Optically Active Pharmaceutical Compounds using Axial Compressed Columns

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A major improvement in preparative chiral column performance has been achieved by adapting axial compression to manufacture Lux® chiral preparative columns. This paper demonstrates the advantages of combining the Lux media and Axia™ packing technology to produce high performance stable preparative chiral columns. This process produces preparative columns packed with 5 µm media with the same efficiency (plates per meter) in prep columns as found in analytical columns and peak symmetry independent of column length and internal diameter.

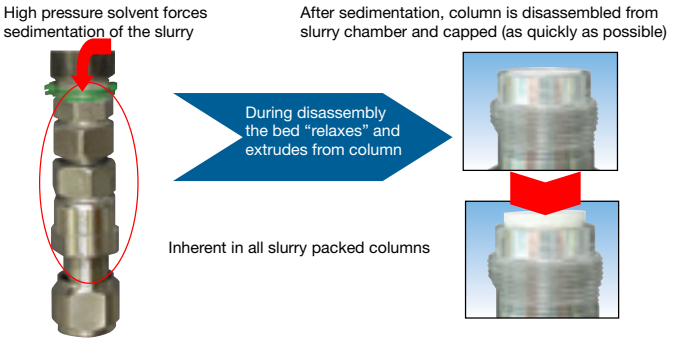
Introduction

Previous limitations with preparative chiral columns

Historically, it has been a limitation of chiral columns that performance and lifetime decrease as the column's internal diameter increases from analytical to preparative dimensions, despite being packed with the same particle size media. Column stability was also inherently less for the preparative chiral columns compared to analytical columns. This lower initial performance and/or loss of performance is inherent in all slurry packed chiral preparative columns, and is caused by:

1. Packed bed structure being disturbed after the media is packed
2. Media fracture, and or fines, created by packing media in large diameter columns
3. Packing density not uniform throughout the column
4. Media extrudes from the packed bed during final hardware assembly (**Figure 1**)

Figure 1.
Conventional Slurry Packed Preparative Chiral Column



With slurry packed columns, the packing hardware must be disassembled before the end fitting is placed on the column. During this procedure the pressure on the media must be released, the packed bed is disturbed and the media begins to extrude from the column creating non-uniform density.

Recent improvements in chiral purification technology

A major improvement in preparative chiral column performance has been achieved by adapting axial compression to manufacture Lux preparative columns. For the last six years the Axia packing technology (explained in **Figure 2**) has been utilized to produce high performance stable achiral preparative columns. This same technology is now employed to produce preparative chiral columns packed with 5 µm chiral stationary phases (CSP).

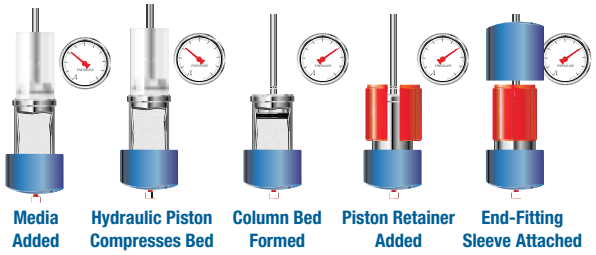
How Axia packed columns perform better

A computerized mechanical process packs the column bed. The force applied to the column is carefully controlled during the packing process to prevent crushing or cracking of the media. The Lux media is engineered to be mechanically stronger than previous chiral media allowing higher packing pressures to be applied (**Figure 3**). Once the column bed forms, the media is never allowed to expand or extrude from the column and the internal packing force is maintained on the column during final hardware assembly.

Creating efficient, more productive chiral purification methods

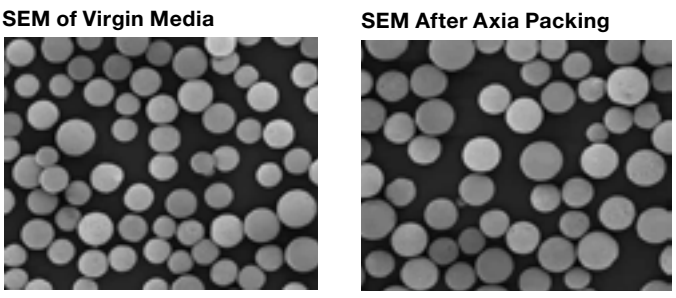
This paper demonstrates the advantages of combining the new Lux media and Axia packing technology to produce high performance stable preparative chiral columns. This process produces preparative columns packed with 5 µm media with the same efficiency (plates per meter) and peak symmetry independent of column length and internal diameter (**Figure 4**). Axia packed preparative columns are manufactured with 5 µm media in 100, 150, and 250 mm lengths with 21.2, 30, and 50 mm internal diameters (**Figure 5**).

Figure 2.
Axia Packing Process Integrates Axial Compression Technology into Prepacked Chiral Preparative Columns



The Axia process uses highly controlled pneumatic mechanical pressure to drive the piston into the column to produce a uniformly packed bed. Once the bed is formed the pressure on the piston and bed is not released, the bed is not disturbed and the piston is locked in place leaving the chiral media under pressure. This packing process won the 2006 R&D 100 Award for its innovation.

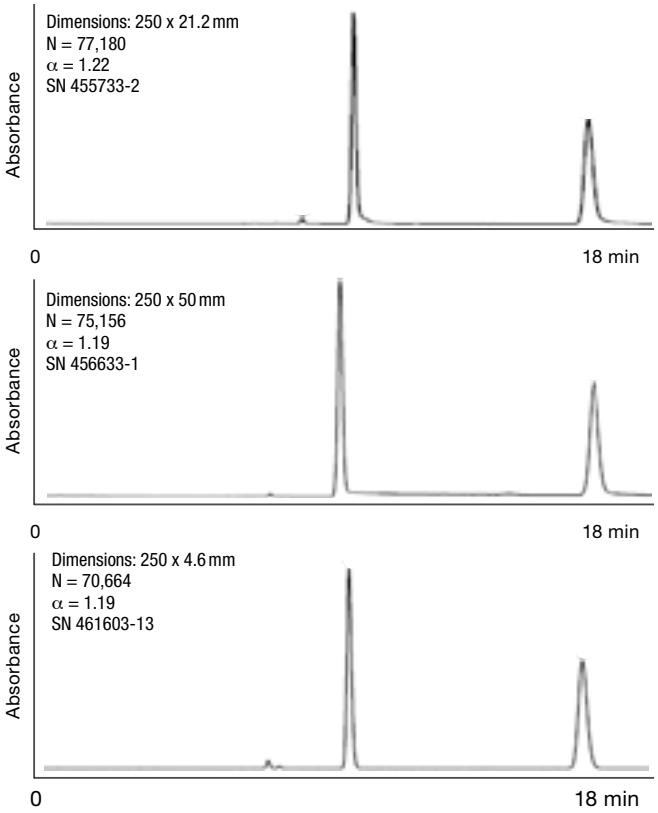
Figure 3.
Controlled Axia Packing Process for Lux Cellulose-2 Prevents Crushing the Media



Lux media is mechanically stronger allowing higher packing pressures than previous chiral media.

The SEM of virgin media and after Axia packing proves that Axia's computer controlled process does not crush the Lux high porosity media that is engineered to be mechanically strong.

Figure 4.
Axia™ Packed Lux® Preparative Columns Provide the Same High Performance Independent of Column Diameter



Conditions for all columns:
Column: Lux 5 µm Cellulose-2
Mobile Phase: Hexane:IPA (90:10)
Detection: UV @ 220 nm
Sample: Trans-Stilbene oxide

Axia technology has the highest process control and produces reproducible, stable, high efficiency columns with the same plates per meter and peak asymmetry independent of column length and ID.

Figure 5.
Axia packed Lux Product Family Available in Three Diameters and Three Lengths

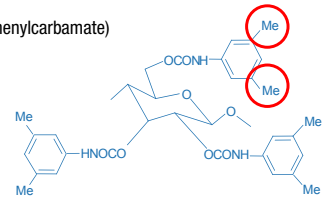
The Axia packing process is utilized to produce the Lux preparative columns in 100, 150 and 250 mm lengths and in the three diameters 21.2, 30 and 50 mm.

Experimental

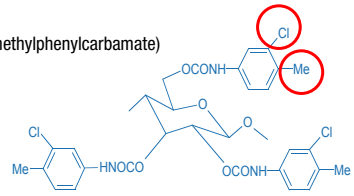
Lux is a media engineered to provide a straightforward approach to enantiomeric recognition and separation by HPLC and Supercritical Fluid Chromatography (SFC). Two Lux phases have been developed using a coated derivatized cellulose material as the chiral selector (**Figure 6**). Lux Cellulose-1 features the classical tris(3,5-dimethylphenylcarbamate) cellulose derivative used industry-wide for many enantiomer separations. This particular chiral selector has well-established enantiomeric abilities to resolve a wide range of racemates.

Figure 6.
Structures of Lux Cellulose-1 and Lux Cellulose-2 Chiral Phases

Lux 5 µm Cellulose-1
Cellulose tris(3,5-dimethylphenylcarbamate)



Lux 5 µm Cellulose-2
Cellulose tris(3-chloro-4-methylphenylcarbamate)

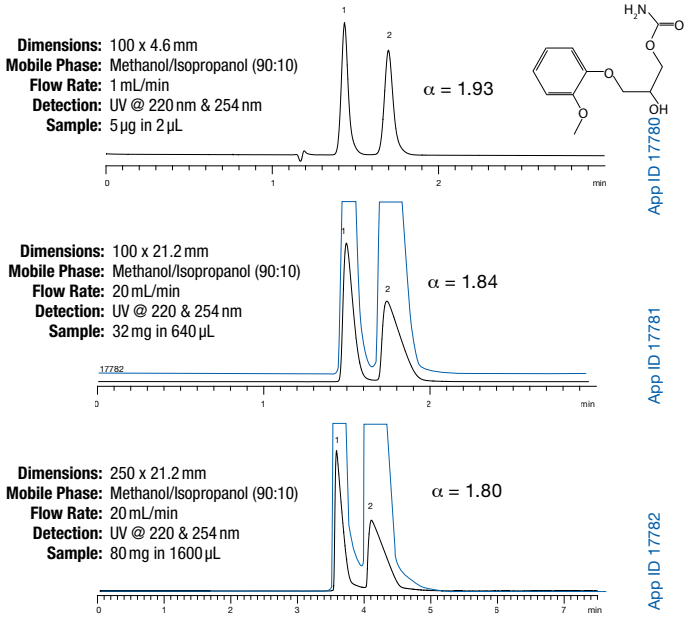


Lux Cellulose-1 and Lux Cellulose-2 are derivatized phenyl carbamates with different functional groups substituted on the aromatic rings. The substitution of the chlorine molecule with Lux Cellulose-2 provides unique selectivity compared to the traditional Lux Cellulose-1 structure.

Lux Cellulose-2 incorporates an advanced halogenated derivative, leading to unique enantioselectivity compared to previously commercialized cellulose phases. The unique selectivity of Lux Cellulose-2 makes it an ideal CSP providing excellent complementary selectivity to Lux Cellulose-1, and any improvement in the alpha value is extremely important for preparative separations. The two Lux phases are compatible with a wide range of solvent systems including normal phase, polar organic, reversed phase, and SFC.

Methocarbamol represents an important class of compounds routinely separated and purified by HPLC with CSP columns. The initial separation was developed on a 100 x 4.6 mm column using MeOH:IPA (90:10) and the response monitored at 220 and 254 nm. Increasing the column diameter from 4.6 to 21.2 mm provides higher throughput (32 mg) for each run without increasing the overall purification time (**Figure 7**). By increasing the column length to 250 mm the load can be further increased to 80 mg per run. The methocarbamol separation demonstrates that the separation scales up linearly based on the column length with no loss of resolution. An important factor to consider when performing these higher mass loading separations is the UV detector response is not linear making it difficult to determine where the major mass is located based solely on the UV signal. Although de-tuning a detector will keep the peaks on scale, the UV signal is not the best indicator for purity and resolution when column overloading occurs. It is crucial to first evaluate the purity and yield for the collected fractions to determine the maximum load per run that could be achieved.

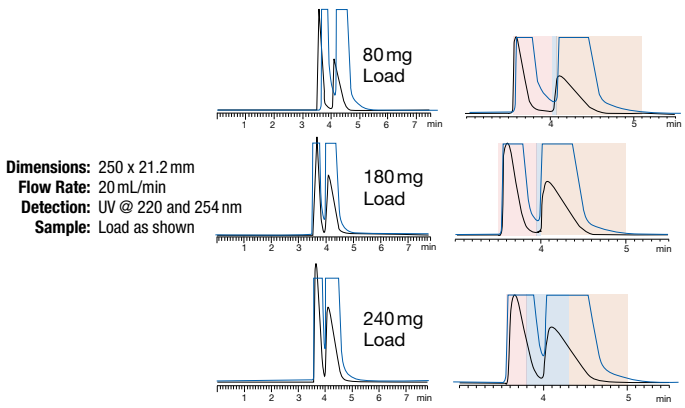
Figure 7.
Direct Scale Up of Methocarbamol Purification on Lux® 5 µm Cellulose-1



Separation scales up directly based on column length. With the 100 mm length column a 32 mg/load separation was achieved and a higher sample load required the longer 250 mm length column. As expected when increasing the load, the peak width and tailing increased but there was no loss of resolution. For the higher sample loads, the detector sensitivity was decreased by monitoring at 254 nm.

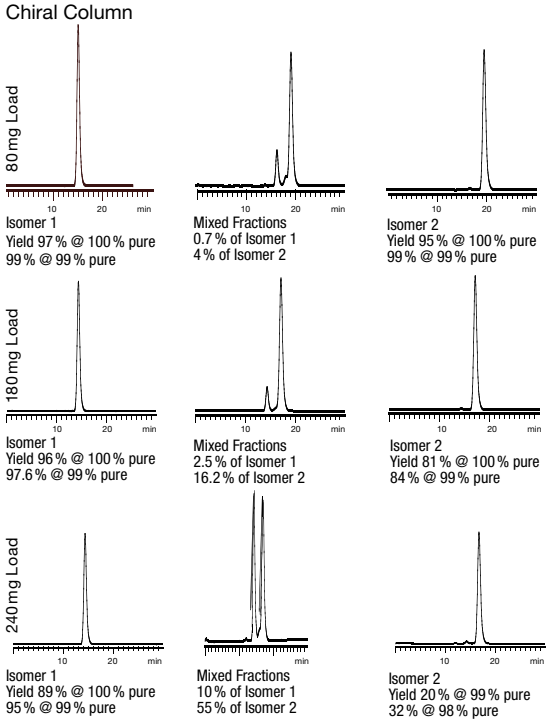
To further improve sample throughput and productivity, the sample load on the column was increased from 80 mg to 180 mg and then finally to 240 mg (**Figure 8a**). The UV signal was used to determine the starting and ending collection points, but time based fractions were collected across the peaks including the valley area. After the fractions were collected, each fraction was first analyzed using a 100 x 4.6 mm Lux Cellulose-1 column on an analytical HPLC and then the fractions were pooled together and the overall yield and purity were assessed (**Figure 8b**).

Figure 8a.
Resolution Change with Increased Load for Chiral Separations



Fractions were collected across the peak and evaluated for purity at 220 nm using a 100 x 4.6 mm Lux Cellulose-1 column. The purity for each pooled fraction was determined. The 254 nm detector trace for the preparative separation shows the presence of each material but cannot be used to predict the purity of the fraction.

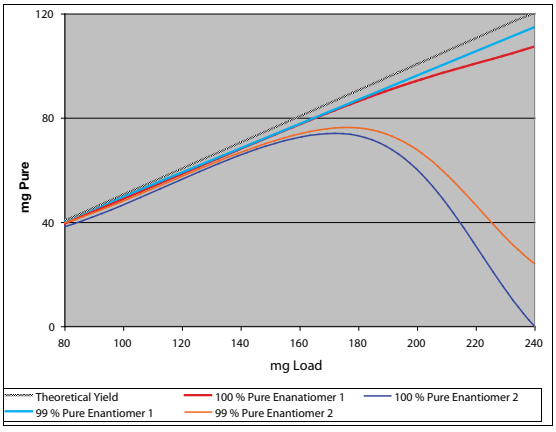
Figure 8b.
Comparison of Yield and Purity for Different Loads on Lux® Cellulose-1 Chiral Column



Each fraction was individually evaluated and then pooled providing three fractions that were then evaluated for the overall purity and yield. At 80 mg load both enantiomer 1 and 2 were obtained in high yield and purity. When 180 mg load was purified, the yield for pure enantiomer 1 was not significantly affected but enantiomer 2's yield decreased to 81 %. With the 240 mg load, the column overload was too great and the capability to collect pure enantiomer 2 was lost. Even if a 2 % enantiomer impurity was acceptable only 32 % of the load was recovered at this purity level.

The dramatic effect increasing sample load has on throughput, purity, and productivity is illustrated in **Figure 9.**

Figure 9.
Effect on Purity and Yield When Increasing Methocarbamol Load to Improve Throughput



The quantity of 100 % pure enantiomer 1 collected increases with larger sample loads with a slight sacrifice in yield. The later eluting enantiomer's purity and yield dramatically drops when the load is above 180 mg on the 250 x 21.2 mm column. A larger diameter column with the same load would provide increased throughput for the second enantiomer without sacrificing purity.

Enantiomer 1 – Even when 240 mg was loaded onto the column, the purity of the first enantiomer was not greatly affected and 107 mg (89 % of this enantiomer) was determined to be 100 % pure. Increasing the mass loading per run is a tremendous advantage when larger quantities of this enantiomer are required. If the enantiomer purity requirement was 99 %, then additional fractions could be pooled and a total of 114 mg (95 %) of the first enantiomer mass was collected.

Enantiomer 2 – When 180 mg was loaded onto the column, 73 mg (81 %) of the enantiomer was obtained at 100 % purity level which is only a slight change from enantiomer 1 results where 86 mg was collected for the same purification. Whereas when the mass loading was increased to 240 mg the purity for enantiomer 2 dramatically decreased. In fact, at the 240 mg load, the highest purity achieved was 99 % for enantiomer 2, but only 24 mg was recovered representing only about 20 % of the initial load. With the 240 mg load, the first enantiomer contaminates the second enantiomer and 0 % was obtained with a 100 % purity level. The extent of the peak overlap is very evident in the preparative chromatograms in **Figure 8a**. Although the UV trace indicates there is still resolution between the two compounds the amount of overlap between enantiomer 1 and enantiomer 2 is very significant. If the desired enantiomer is the later eluting compound on the chiral column, the sample load and throughput must be carefully monitored by evaluating the fractions to ensure an acceptable enantiomer purity is achieved.

Conclusions

Axia technology is the industry standard for consistency and robustness in preparative columns with the same performance achieved from 4.6 mm analytical columns to 50 mm ID preparative columns. Over the last several years the Axia technology with its high level of process control has been proven to produce columns with the same performance (plates per meter) independent of length and diameter. The 5 µm Lux preparative columns are available in 100, 150, and 250 mm lengths with 21.2, 30, and 50 mm diameters. There has been a significant improvement in the asymmetry and efficiency across all lengths and IDs for the Axia packed preparative columns allowing chemists more flexibility to achieve their goals for increased purity and yield for their preparative purifications.

Since these preparative Lux columns packed with 5 µm have the same plates/meter (efficiency and asymmetry factors) independent of ID and length, the chemist has more options to quickly scale up a separation to obtain higher quantities of purified enantiomer without sacrificing purity or yield. Many times the shorter column provides sufficient resolution for the required compounds resulting in faster turn-around times and higher productivity.

Selectivity is still the most critical factor for chiral separations. Screening and having a choice of multiple chiral phases is important to increase resolution prior to performing the preparative separations.

This work has shown that sample load scales up directly based on column length, but the separation time increases as the column length increases. Whereas, sample load increases exponentially with column diameter without increasing the separation time. The judicious choice of overall column length and column diameter of the Axia packed Lux columns will have a major affect on a laboratory's overall throughput.

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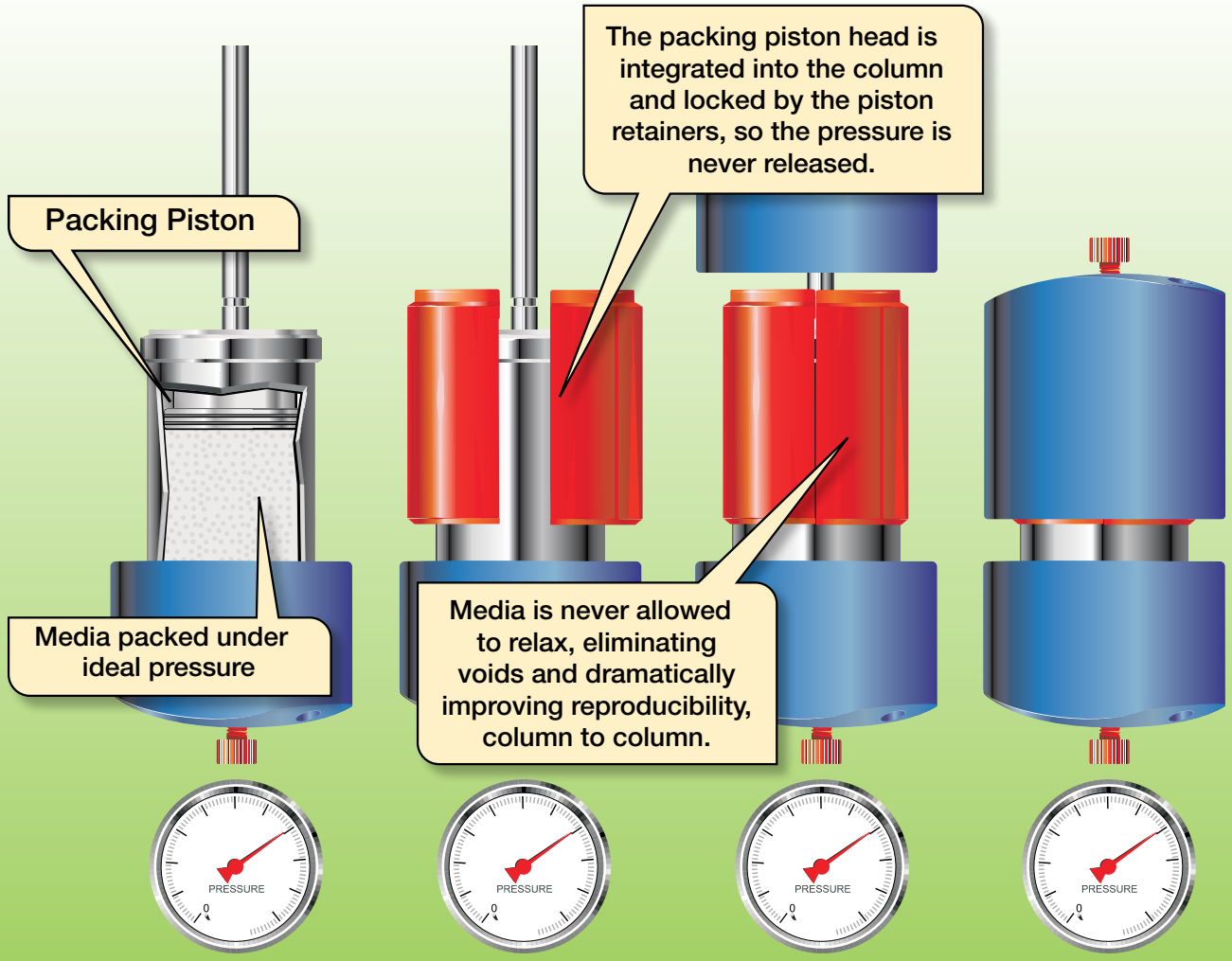

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Direct Comparison of HPLC and SFC for the Milligram to Gram Scale Purification of Enantiomers

Philip J. Koerner[1], Peter C. Rahn[1], Gary W. Yanik[2], and Irene Tranquil[2]
[1] Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA
[2] PDR-Chiral Inc. 1331A South Killian Drive, Lake Park, Florida 33403 USA

High efficiency Axia™ preparative columns packed with Lux® polysaccharide-based chiral stationary phases are utilized to compare chiral SFC and HPLC purification of several pharmaceutical compounds.

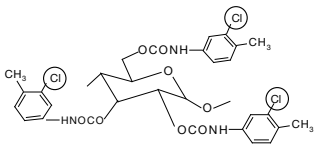
Introduction

High-throughput analysis and purification of enantiomers are important in drug discovery. With today's regulations to improve safety and efficacy of drugs, the pharmaceutical industry needs to provide high quality pure enantiomers for pharmacological testing. Historically, chiral purification has been achieved with normal phase HPLC, and more recently, with reversed phase separations. In recent years, SFC has gained acceptance as a very effective complementary tool for other chiral separation modes to produce pure enantiomeric compounds.

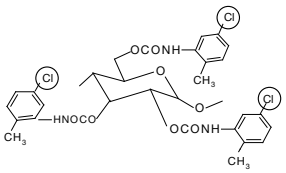
This technical note compares the throughput (grams per hour) for the same chiral preparative separations performed under HPLC conditions and SFC conditions. In this work, three different polysaccharide-based chiral stationary phases (CSPs) are utilized (**Figure 1**): Lux Cellulose-1, Lux Cellulose-2, and Lux Amylose-2. The same 5 µm media was used to produce both the analytical columns and the Axia packed preparative columns. This work also demonstrates the capability to directly scale analytical chiral separations to preparative chiral separations when both column sizes are packed with 5 µm media.

Figure 1.
Structures of Polysaccharide-based Chiral Phases

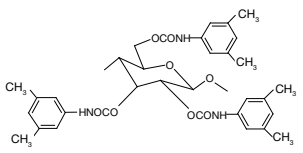
Lux® Cellulose-2
Cellulose tris
(3-chloro-4-methylphenylcarbamate)



Lux® Amylose-2
Amylose tris
(5-chloro-2-methylphenylcarbamate)



Lux® Cellulose-1
Cellulose tris
(3,5-dimethylphenylcarbamate)

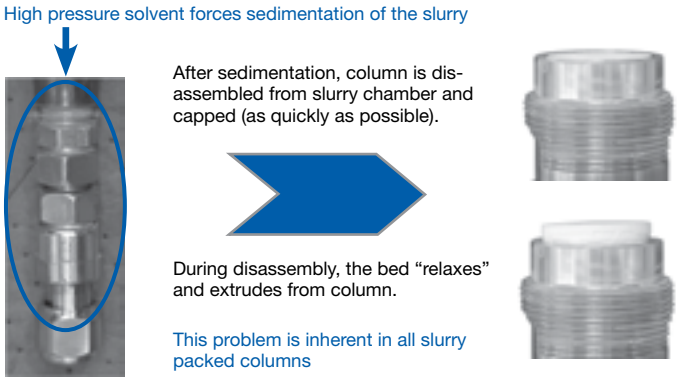


Background

In the past, the same particle size chiral media packed in 4.6 mm ID columns was packed into preparative columns but the column performance and lifetime decreased as the column internal diameter increased. Column stability was also inherently less for the preparative chiral columns compared to analytical columns. The lower initial performance and/or loss of performance are inherent in all slurry packed chiral preparative columns and caused by:

1. Packed bed structure being disturbed after the media is packed
2. Media fracture, and or fines, created by packing media in large diameter columns
3. Non-uniform packing density throughout the column
4. Media extrusion from the packed bed during final hardware assembly (**Figure 2**).

Figure 2.
Conventional Slurry Packed Preparative Chiral Column



A major improvement in preparative chiral column performance has been achieved by adapting Axia packing technology to manufactured Lux chiral preparative columns. Axia packing technology (**Figure 3**) has been utilized to produce stable, high performance achiral preparative columns. This same technology is now employed to produce preparative chiral columns packed with 5 µm chiral stationary phases. The Lux media is engineered to be mechanically stronger than previous chiral media, allowing higher packing pressures to be applied. A computerized mechanical process packs the column bed. The force applied to the column is carefully controlled during the packing process to prevent crushing or cracking of the media. Once the column bed forms, the media is never allowed to expand or extrude from the column and the internal packing force is maintained on the column during final hardware assembly. The advantages of combining the new Lux media and Axia packing technology to produce high performance stable preparative chiral columns are

illustrated in this technical note. The Axia packing technology produces preparative columns packed with 5 µm media having the same efficiency and peak symmetry as analytical columns (**Figure 4**). Axia packed preparative columns are manufactured with Lux 5 µm chiral media in 100, 150 and 250 mm lengths with 21.2, 30 and 50 mm internal diameters.

Figure 3.
Axia™ Packing Process Integrates Axial Compression Technology into Pre-packed Chiral Preparative Columns

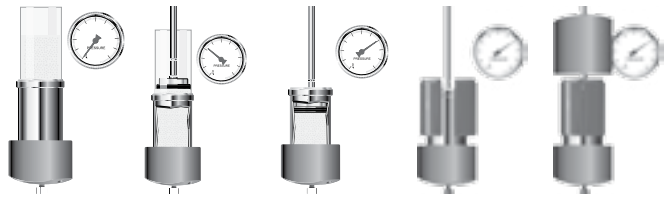
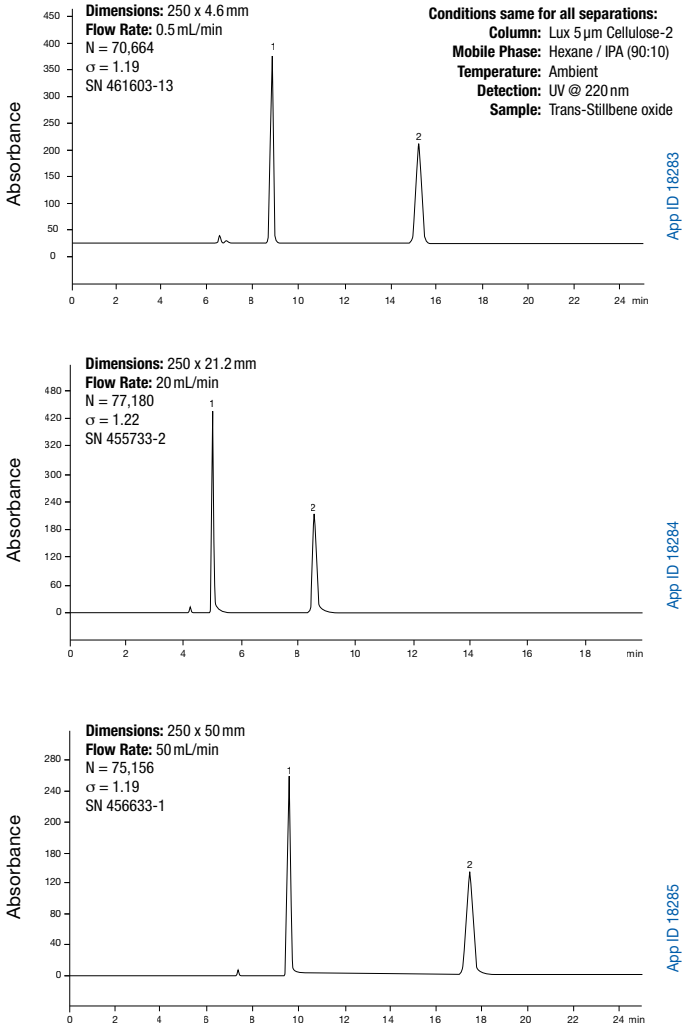


Figure 4.
Axia™ Packed Lux® Preparative Columns Provide the Same High Performance Independent of Column Diameter



Experimental Conditions

Analytical HPLC separations were developed using an Agilent® 1100 system with diode array detector (Agilent, Palo Alto, CA). The Gilson 845ZPREP™ HPLC system (Gilson, Middleton, WI) was used for the preparative HPLC separations and fraction collection. For SFC separations, a Berger preparative SFC system was utilized consisting of the pumping system, variable UV and PDR-Chiral detectors, and a 6-port fraction collector capable of collecting hundreds of milliliters of eluent. The advanced laser polarimeter (ALP) detector (PDR-Chiral, Lake Park, FL) measures the rotation of plane-polarized 660 nm laser beam passing through the flow cell and indicates the optical rotation of each enantiomer. Using a mobile phase consisting of 25 % polar modifier (methanol or ethanol) added to the carbon dioxide (CO_2), a flow rate of 50 mL/min through the 21.2 mm diameter columns was easily achieved without exceeding the 200 bar pressure limits of the SFC instrument.

Results and Discussion

Figures 5A – 5D contain the SFC and HPLC chromatograms, purification conditions, and results for each sample. **Figure 5A** compares the Atenolol separation for SFC and HPLC. The SFC conditions required 25 % methanol while the HPLC conditions yielded the best separation with 20 % ethanol. The PDR-Chiral detector (ALP) indicates the optical rotation of each enantiomer. The SFC cycle time was 4 minutes and the HPLC cycle time was 6 minutes. In addition to a faster cycle time the total load on the column was also 1.8 times higher for SFC (102 mg for SFC vs. 60).

Figure 5B compares the Terfenedine HPLC and SFC separations. The Lux Cellulose-1 column using 25 % methanol as the polar modifier provided the best SFC separation. The Lux Cellulose-1 column with the polar organic mobile phase of 3 % isopropanol and 97 % acetonitrile provided the best HPLC separation. SFC sample load per cycle was 105 mg compared to 12 mg/cycle for the HPLC runs in the same 7 minute cycle time for SFC and for HPLC. This results in a significantly higher throughput for SFC (840 mg/hour) compared with HPLC (102 mg/hour) and a significantly smaller volume collected per gram of product. The overall purity and recovery for HPLC and SFC were the same.

Figure 5C compares the two Propranolol chiral separations. For SFC, the Lux Cellulose-1 column with 25 % methanol polar modifier provided the best separation with a sample load of 120 mg per cycle. The HPLC conditions also utilized the Lux Cellulose-1 column with 20 % isopropanol as the polar solvent (hexane as the non-polar solvent) and a sample load of 60 mg per cycle. Since the load per cycle was higher for SFC, the throughput for SFC was 450 mg/hour compared to 553 mg/hour for HPLC and the total volume collected for 1 gram of product was 508 mL for SFC compared to 799 mL for HPLC.

The Propafenone separations using Lux Amylose-2 are shown in **Figure 5D**. Lux Cellulose-1 and Lux Cellulose-2 could not resolve these enantiomers in HPLC or SFC but a 50 mm long Lux Amylose-2 provided the best resolution possible although the resolution is on the low end for a preparative purification. The SFC conditions utilized 20 % ethanol as the polar solvent with a load of 15 mg per 7 minute cycle. Under HPLC conditions, 50 % isopropanol was required to achieve the separation on the Lux Amylose-2 column with a load of 18 mg per 4 minute cycle. The throughput is limited by the resolution between the two compounds, which is minimal

Conclusions *(cont'd)*

Advantages of SFC relative to HPLC are dependent on the analyte, the CSP, and the amount of polar solvent required. Generally, higher load and higher flow rates were achieved with SFC but the volume collected per gram of product was not as different as other achiral separations previously reported. This is a result of the higher percentage of polar mobile phase solvent used in the SFC separations.

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Use different chiral column selectivities to develop more efficient methods.

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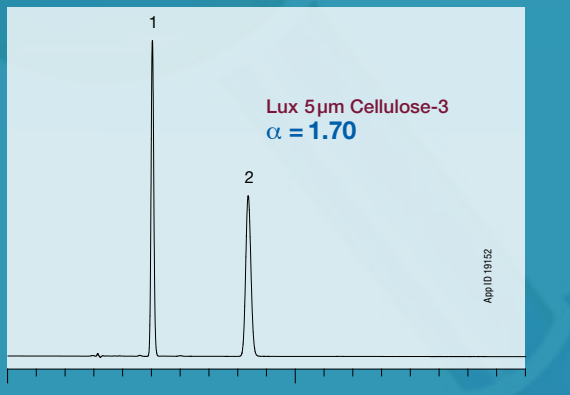
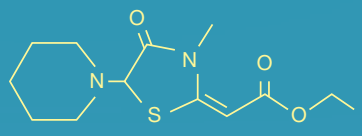


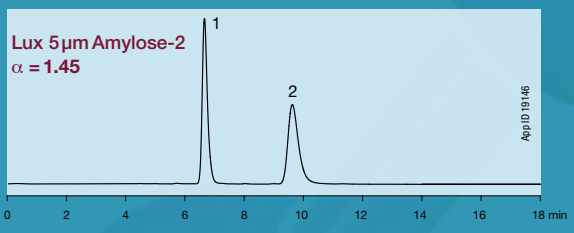
Based on a five phase screen under reversed phase conditions, the optimal chiral stationary phase for resolving Etozolin is Lux Cellulose-3.

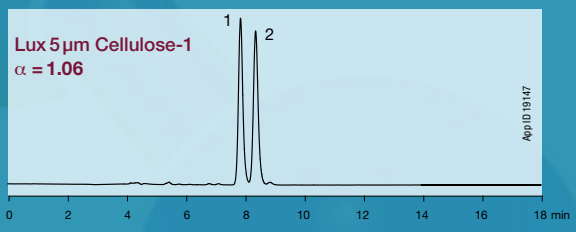
Etozolin

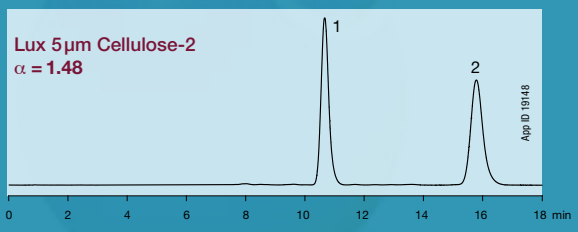


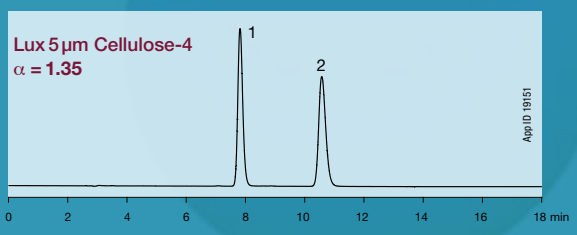
Conditions for all columns:

Column:	As noted
Dimension:	250 x 4.6 mm
Mobile Phase:	Acetonitrile / 20 mM Ammonium bicarbonate with 0.1 % Diethylamine (60:40)
Flow Rate:	1 mL/min
Temperature:	Ambient
Detection:	UV @ 220 nm









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Scaling from Analytical to Preparative Chiral Chromatography While Balancing Purity, Yield, and Throughput under HPLC and SFC Conditions

J Preston, J.T. Presley III, Michael McCoy, Michael Klein, Marc Jacob et al.
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Axia™ preparative column technology along with Axia specilized hardware shows higher performance than traditionally packed standard hardware preparative columns. The Axia packing technology is compatible with both SFC and HPLC conditions. In this application, we will demonstrate how the Axia packed columns with the Lux® Cellulose-1 polysaccharide-based chiral stationary phase can be a tool to increase throughput for purification of chiral compounds.

Introduction

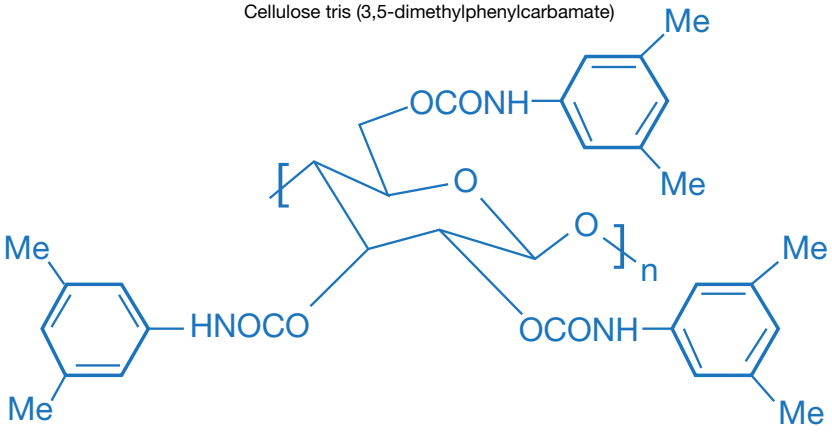
HPLC has been extensively studied since the late 1960's and there have been numerous theoretical models developed to describe, explain, and predict the results of chromatographic experiments. The typical goal of chromatography is to separate compounds from each other, and the most straight forward way to evaluate a separation is to calculate the resolution between two peaks of interest. Resolution of two peaks will be a function of numerous factors, including mobile phase composition, stationary phase selectivity, and running conditions. In practical terms, the resolution is predicted by how far apart the two peaks are separated in time and how broad the peaks are shaped. Thus, optimal resolution is provided by obtaining narrower peaks, as this allows them to be more easily resolved from one another in any given time frame.

One particular method of chromatography known as supercritical fluid chromatography (SFC) has become increasingly popular in the last several years. In contrast to traditional liquid chromatography, the SFC mobile phase consists of a mixture of liquid carbon dioxide and organic solvent, such as Methanol. The principle advantages of SFC over conventional HPLC techniques are increased speed, reduced waste generation and for preparative purifications, minimized post-chromatography sample manipulation. For chiral separations in particular, SFC is increasing in popularity because it is often very simple to convert an existing normal phase HPLC method into an SFC method. The use of preparative chiral chromatography has increased significantly over the past 5-10 years, and SFC has been a significant driver for this increase.

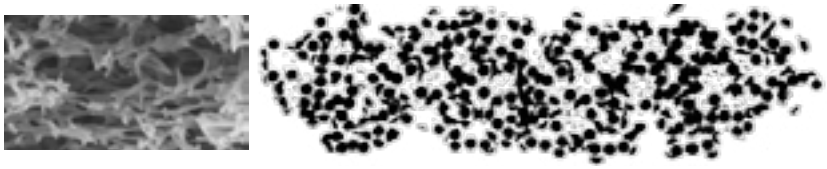
It is well known that chromatography can be directly scaled from very small columns to very large columns when the eluent composition remains consistent. The work presented in this application will address the relationship between both normal phase and SFC chiral methodologies at the analytical and preparative scale. The impact on resolution at both scales due to flow rates will be evaluated and compared between SFC and normal phase. The effect of preparative column hardware technology along with resulting purity and throughput from related SFC and normal phase purification methodologies will also be evaluated.

Lux Cellulose-1 Chiral Stationary Phase

Cellulose tris (3,5-dimethylphenylcarbamate)



Cellulose backbone



Material and Methods

Analytical HPLC separations were developed using an Agilent® 1100 system with diode array detector (Agilent, Palo Alto, CA). SFC analytical was performed on a Waters® ACQUITY® UPC²® system (Waters, Milford, MA USA) consisting of a convergence manager, sample manager, binary solvent manager, PDA detector, column manager with 6 positions, and a Waters 3100 mass spectrometer. Data analysis was performed using MassLynx® software (Version 4.1).

Normal phase preparative scale separations were performed on a Shimadzu® LC20 Prep HPLC system, with an LC-10 autosampler and fraction collector. SFC purifications were performed on a Berger Automated PrepSFC™ system (Mettler-Toledo, USA) consisting of a Bohdan automated injection/collection robot, Berger SCM-250 (separator control module), Berger ECM-2500 (electronic control module), KNAUER K-2500 UV variable detector, Varian® SD-1 methanol and CO_2 delivery systems, JULABO® chiller, and SFC ProNTo™ control software (Version 1.5.305.15) with SFC Automation Controller add-on (Version 1.5.92.3).

Compounds were evaluated using a Phenomenex Lux® 5 µm Cellulose-1 column, dimensions are as noted in each Figure. HPLC conditions and injection amounts are as noted in each Figure. Warfarin test solutions were prepared at 20 mg/mL in ethanol and used for all testing.

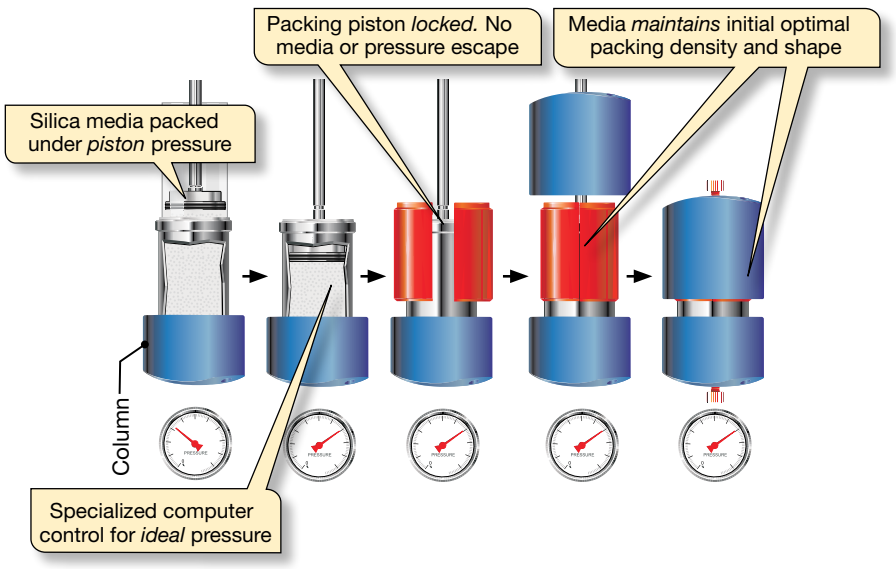
Results and Discussion

When scaling up from analytical sized columns (4.6 mm I.D.) to traditionally packed larger I.D. columns (>10 mm I.D.), there has historically been some loss in efficiency and performance that is attributed to the packing quality with these larger I.D. columns. The reasons for this change in performance are complex but include non-uniform packing density throughout the column, the bed structure being disturbed after the media is packed, media fracture and/or fines created during the packing process, and media extrusion from the packed bed during final hardware assembly.

The standard hardware column packing process is complicated and there are many opportunities for a loss in column performance. To address this issue, Phenomenex developed a unique column packing technology and hardware, AXIA™, to maintain analytical-like column performance in preparative column dimensions. The Axia technology, patented by Phenomenex, is an advanced column packing and column hardware design that incorporates Hydraulic Piston Compression technology that mimics axial compression columns. This results in Axia preparative columns outperforming column packed using traditional packing methods.

Axia packing technology uses a computerized mechanical process to pack the column bed (**Figure 1**). The force applied to the column is carefully controlled during the packing process to prevent crushing or cracking of the media. Once the column bed forms, the media is never allowed to expand or extrude from the column and the internal packing force is maintained on the column packing during final hardware assembly and into the final product.

Figure 1. Axia Patented Packing Technology



Previous work by Jan Priess et. al. demonstrated increased column efficiency and resolution for polysaccharide-based chiral stationary phase (CSP) media packed using Axia™ columns.[1] To better understand how much this hardware technology improves column performance we packed the same 5 µm Lux® Cellulose-1 chiral media into two different 150 x 21.2 mm I.D. columns. The Lux media is engineered to be mechanically stronger than previous chiral media, allowing higher packing pressures to be applied; thus increasing the column plate count and column performance. One column was packed using a traditional HPLC column packing process with standard hardware and the other column was packed using Axia technology with Axia hardware. The QC data for the Axia column showed 73,000 plates per meter, which was a > 22 % increase over the standard hardware column.

The 150 x 21.2 mm traditionally packed standard hardware preparative column and Axia packed preparative column were first evaluated by generating Van Deemter curves for trans-Stilbene Oxide (TSO) to find out if there was any difference in column efficiency versus linear velocity. The normal phase data indicated the Axia packing technology had a substantial 91.6 % increase in column efficiency over traditionally packed columns at a 0.1 cm/sec linear flow as depicted in **Figure 2**. The difference in performance was less pronounced in SFC, but still showed a 26.8 % increase in efficiency for the Axia packed column at 0.4 cm/sec (**Figure 3**). As expected, the decrease in column efficiency as linear velocity increased was less under SFC conditions.

Figure 2. Van Deemter Plots - Normal Phase Mode

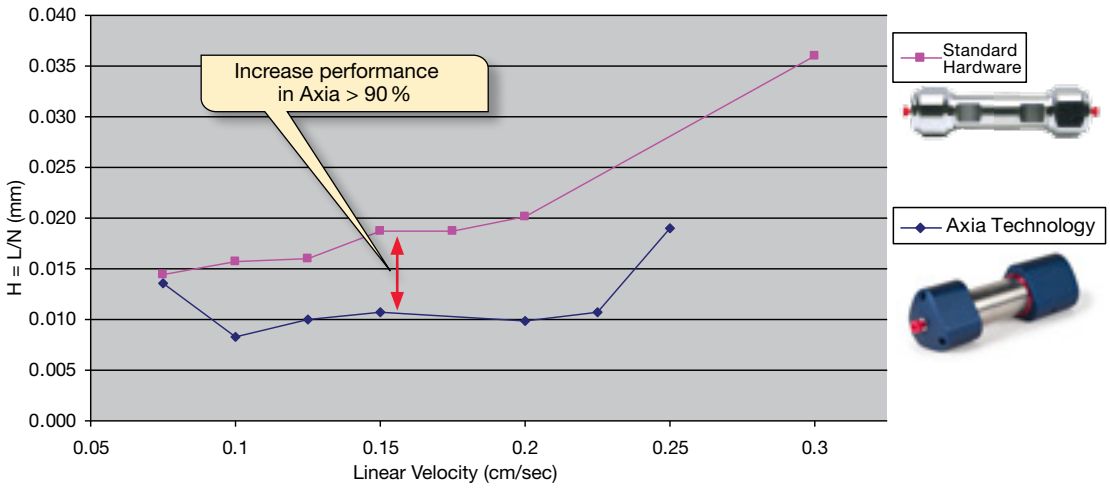
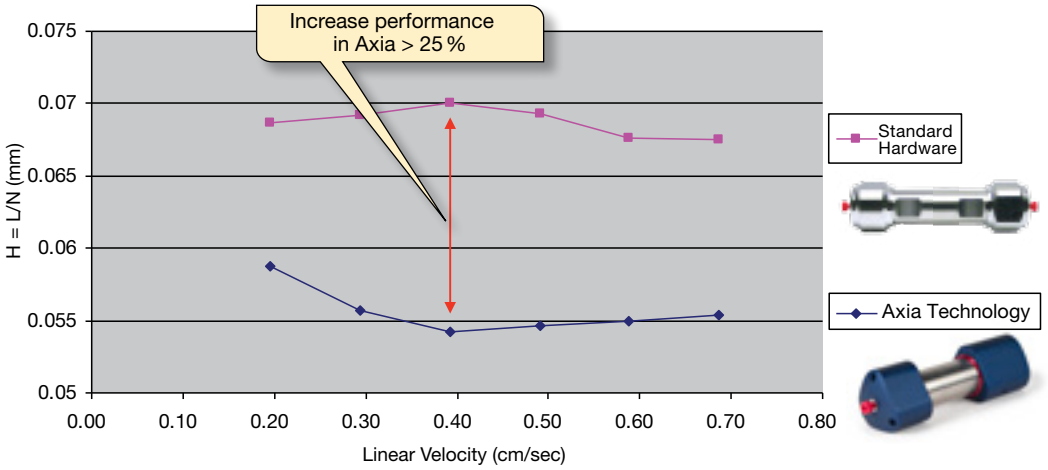


Figure 3. Van Deemter Plots - SFC Mode



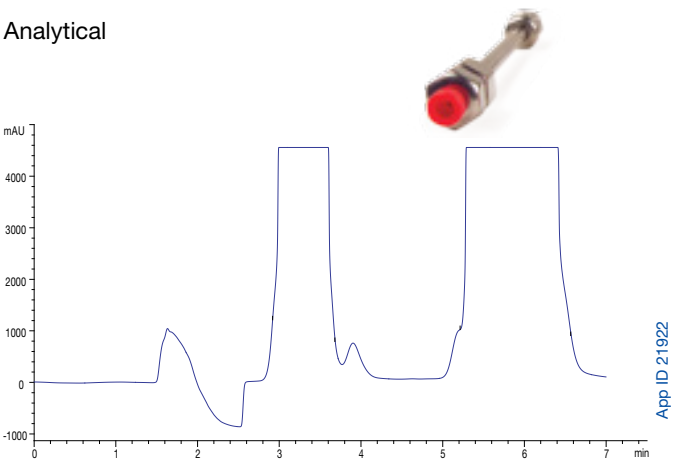
To understand what advantage this would provide for a high-throughput purification laboratory, we performed a scale up experiment using Warfarin. Analytical separations were first developed in normal phase on a 150 x 4.6 mm column and loading was increased until a reasonable loading capacity was achieved. The injection volume was then directly scaled up (geometrically) twenty fold for both of the 150 x 21.2 mm preparative columns. The resolution and efficiency for the second peak were measured. Again, the preparative column packed using Axia™ technology showed roughly a 30 % increase in resolution and 42 % increase in efficiency over the traditionally packed standard hardware column. (**Figure 4**).

Figure 4.
Warfarin Purification in Normal Phase Mode

Column (mm)	Analytical 150 x 4.6	Standard 150 x 21.2	Axia 150 x 21.2
Mass Loaded (mg)	2	40	40
Resolution*	1.5	2.85	3.72
Plates (N)	117	535	760

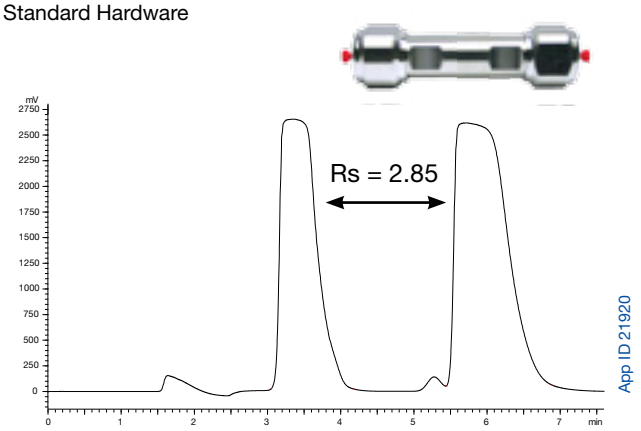
* Resolution calculated with peak width at baseline and center retention time due to the overloaded peaks being off-scale

Analytical

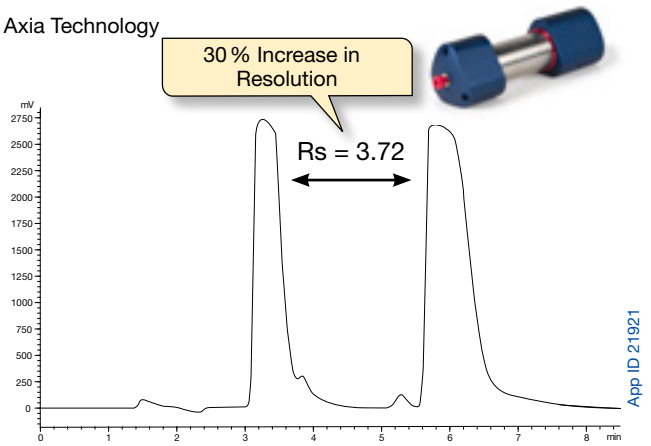


Column: Lux® 5 µm Cellulose-1
Dimensions: 150 x 4.6 mm
Mobile Phase: Hexane/Ethanol (75:25)
Flow Rate: 1 mL/ min
Temperature: Ambient
Inj. Volume: 100 µL

Standard Hardware



Axia Technology



Conditions for both columns:
Column: Lux 5 µm Cellulose-1
Dimensions: 150 x 21.2 mm
Mobile Phase: Hexane / Ethanol (75:25)
Flow Rate: 20 mL/ min
Temperature: Ambient
Inj. Volume: 2 mL

The separation was then adapted to SFC to provide a reduced run time, while maintaining suitable resolution. Due to the SFC systems injection volume limitation, direct loading comparisons were not possible. We did attempt to make a more concentrated solution of Warfarin, but reached a saturation point. However, when comparing loads of 36 mg on-column, the Axia™ columns again showed a 25 % increase in resolution and a 14 % increase in efficiency for the second peak (**Figure 5**) when compared with the traditionally packed standard hardware column.

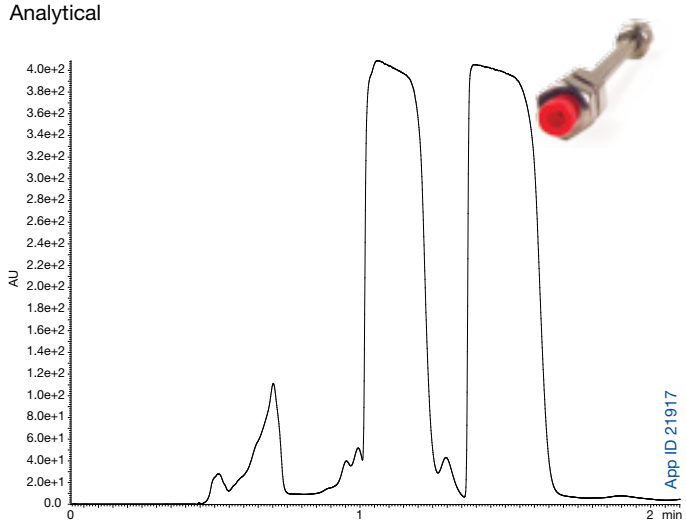
Fractions were collected for both normal phase and SFC runs and yielded similar masses collected with similar purity profiles. This was to be expected since the peaks were still well resolved at this load.

Figure 5.
Warfarin Purification in SFC Mode

Column (mm)	Analytical 150 x 4.6	Standard 150 x 21.2	Axia 150 x 21.2
Mass Loaded (mg)	1.5	36	36
Resolution*	1.39	1.87	2.33
Plates (N)	206	441	503

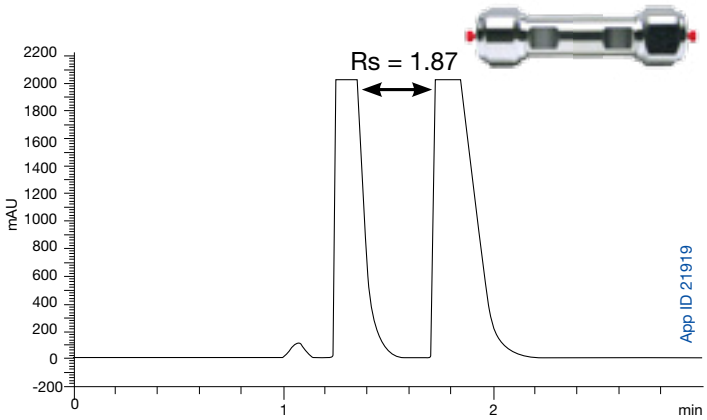
* Resolution calculated with peak width at baseline and center retention time due to the overloaded peaks being off-scale

Analytical

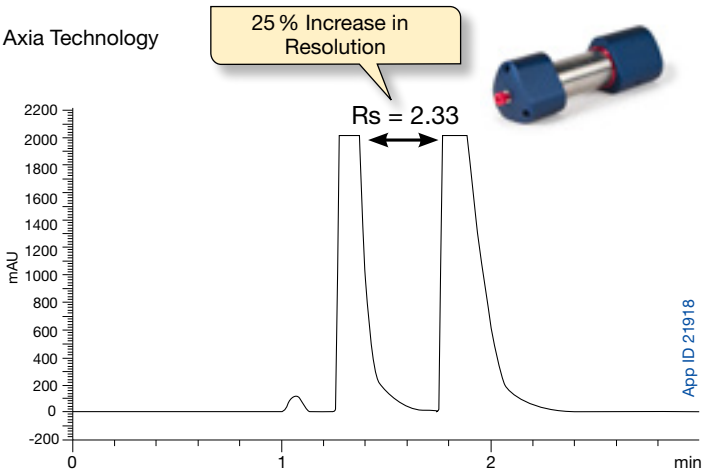


Column: Lux® 5 µm Cellulose-1
Dimensions: 150 x 4.6 mm
Mobile Phase: CO_2/Methanol (65:35)
Flow Rate: 3.5 mL/min
Temperature: 55 °C
Inj. Volume: 75 µL

Standard Hardware



Axia Technology



Conditions for both columns:
Column: Lux 5 µm Cellulose-1
Dimensions: 150 x 21.2 mm
Mobile Phase: CO_2/Methanol (65:35)
Flow Rate: 70 mL/min
Temperature: 55 °C
Inj. Volume: 1.8 mL

Conclusion

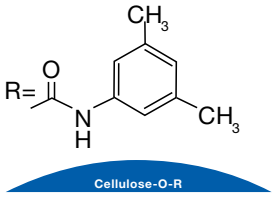
Axia™ preparative columns packed with 5 µm Lux® polysaccharide-based media gives higher performance than traditionally packed standard hardware columns. The Axia technology is compatible with both SFC and HPLC separation conditions and can be a tool to increase throughput for purification.

References

1. Evaluation of Chiral Stationary Phase Packed Axia HPLC Column, J. Priess, C. Valente, G. Diehl and E. Francotte, Novartis Institutes for Biomedical Research, Basel, Switzerland, Poster 2137, SPICA 2008

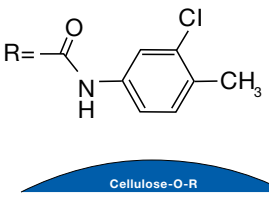
Acknowledgements

The authors would like to acknowledge Bill Ferrell at Pfizer, La Jolla, CA for his contribution to this work.



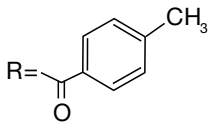
Cellulose-O-R

Lux Cellulose-1
Cellulose tris (3.5-dimethylphenylcarbamate)



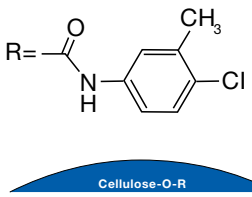
Cellulose-O-R

Lux Cellulose-2
Cellulose tris (3-chloro-4-methylphenylcarbamate)



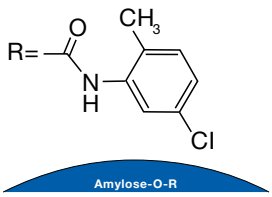
Cellulose-O-R

Lux Cellulose-3
Cellulose tris (4-methylbenzoate)



Cellulose-O-R

Lux Cellulose-4
Cellulose tris (4-chloro-3-methylphenylcarbamate)



Amylose-O-R

Lux Amylose-2
Amylose tris (5-chloro-2-methylphenylcarbamate)

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Pharmaceutical Drugs Applications

page

Enantiomeric Separation of Proton Pump Inhibitors Including Rabeprazole and Pantoprazole Using Lux® Polysaccharide-Based Chiral Stationary Phases in Reversed Phase Conditions

Michael McCoy, Kari Kelly, Marc Jacob and Jeff Layne
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Abstract
We demonstrate in this technical note the successful separation of protein pump inhibitors (PPI) Omeprazole, Lansoprazole, Rabeprazole and Pantoprazole using Lux polysaccharide-based columns. These chiral separations indicate the potential to prepare enantiomerically pure forms of racemic active ingredients Pantoprazole (Protonix) and Rabeprazole (AcipHex) using chiral chromatography.

Introduction
The competition for market share between PPI is fierce. Among therapeutic agents, PPI accounted for $13.6 billion of the total $300.3 billion in sales for the year 2009[1]. In terms of total United States prescription revenues, the PPI Nexium[2] is second only to Lipitor, a member of the drug class known as statins, used for lowering blood cholesterol.

Esomeprazole **1**, the active ingredient for the drug Nexium is the S enantiomer form of Omeprazole **2**, the active ingredient for the drug Prilosec. The patent for Prilosec, which posted sales of $5.6 billion in 2001, expired in 2002. Generic Omeprazole may have eroded the market share of Prilosec significantly had it not been for the chiral separation, subsequent asymmetric synthesis, and timely marketing of Esomeprazole. By the end of 2002 combined sales of Nexium and Prilosec were nearly $6.6 billion.

This marketing patent-loss pattern has also been demonstrated with the introduction of Dexlansoprazole **3** (Kapidex) which is the R enantiomer of Lansoprazole **4** (Prevacid) and which was approved by the FDA in 2009 corresponding to the expiration of patent protection for the drug Prevacid.

A chiral screen was performed on Phenomenex Lux polysaccharide-based columns to identify chiral stationary phases (CSP) for possible preparative scale separation of the enantiomers of four benzimidazoles: Omeprazole **2**, Lansoprazole **4**, Rabeprazole **5** and Pantoprazole **6** under conditions suitable for mass spectroscopy (MS) detection. Optimization of the chromatographic conditions with respect to retention, enantioseparation, and resolution was achieved by variation of the mobile phase constituents at room temperature. The structures of the four analytes are depicted in **Figure 1**.

Materials and Methods
All Analyses were performed using an HPLC Agilent® 1100 series (Agilent Technologies, Palo Alto, CA, USA).

Chiral chromatographic separations follwed by UV detection were performed using Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 HPLC columns with dimensions 250 mm x 4.6 mm ID packed with 5 µm particles (Phenomenex, Torrance CA USA).

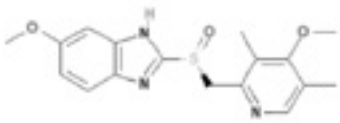
The system flow rate was set to 1 mL/min and the column temperature was ambient. The mobile phases consisted of acetonitrile or methanol with 0.1% diethyl amine (DEA) (solvent A) and 20 mMoL ammonium bicarbonate with 0.1% DEA (solvent B).

Results and Discussion
Five different polysaccharide-based chiral stationary phases Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 were explored in the reversed phase elution mode for the enantioseparation of Omeprazole **2**, Lansoprazole **4**, Rabeprazole **5** and Pantoprazole **6** using mobile phases consistent with LC/MS detection.

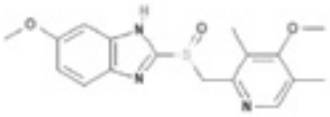
In order to reduce the volume of solvents used, the screening procedure was initially done on Lux 5 µm columns with dimension of 150 x 4.6 mm. After the screening identified the best Lux polysaccharide phases, mobile phase conditions were further optimized on 250 mm length columns of the same particle size and column internal diameter. The best results are shown in **Figures 2-5**; Rabeprazole **5** and Lansoprazole **4** show optimal resolution on Lux Cellulose 4 whereas Omeprazole **2** and Pantoprazole **6** are best resolved on Lux Cellulose 2 phase.

Figure 1. Structures of Analytes

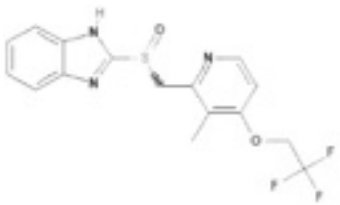
1. Esomeprazol (Nexium)



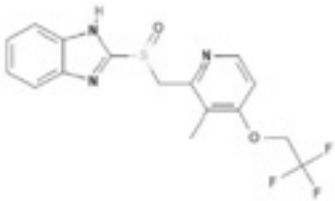
2. Omeprazole (Prilosec)



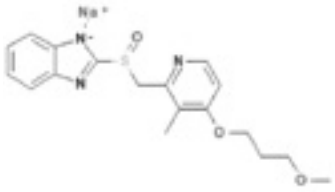
3. Dexlansoprazole (Kapidex)



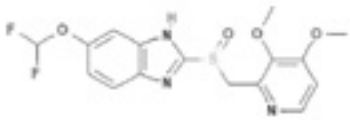
4. Lansoprazole (Prevacid)



5. Rabeprazole (Aciphex)



6. Pantoprazole (Protonix)



Basic or acidic mobile phase additives are often required for improving resolution and peak shapes of ionisable analytes. Aqueous mobile phase buffer at higher pH with ammonium salts such as acetate or hydrogencarbonate (with ammonia) can be effective in the chiral separation of basic racemic compounds. Ammonium salts are thermolabile, hence fully compatible with MS detectors, and even amenable to preparative purifications (as they can be easily removed from the final product).

The chromatogram for the racemic Lansoprazole **4** is shown in **Figure 2a**; while **Figure 2b** confirms the indentify of the single enantiomer of Lansoprazole as Dexlansoprazole.

Likewise, the chromatogram in **Figure 3b** confirms the identity of the single enantiomer of Omeprazole as Esomeprazole. The chromatogram for the racemic Omeprazole is shown in **Figure 3a.**

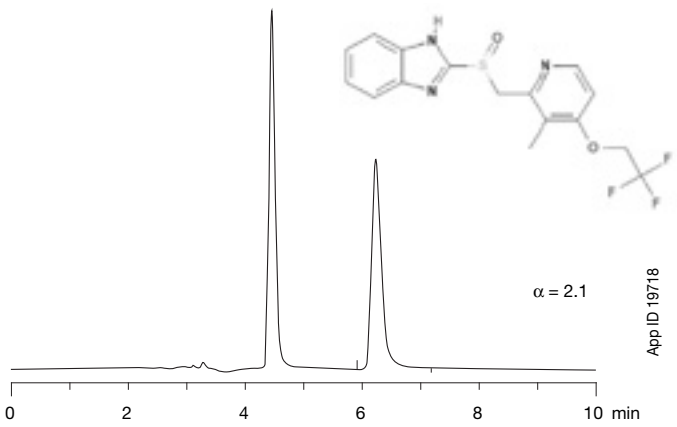
Pantoprazole and Rabeprazole chromatograms shown in **Figures 4** and **5** are racemic mixes. The Lux Cellulose-2 and Lux Cellulose-4 columns, respectively, provide more than enough enantioselectively to allow for chromatographic separation. This suggests that it would be possible to isolate entiomerically pure compounds using Lux polysaccharide-based chiral columns.

Conclusion

The HPLC analysis of the four benzimidazoles Omeprazole, Lansoprazole, Rabeprazole and Pantoprazole allows for fast and accurate identification of their enantiomers. The proven success of marketing enantiomeric pure pharmaceutical ingredients (such as Esomeprazole and Dexlansoprazole) after expiration for the patent of their racemic formulations suggests that chiral purification of Pantoprazole and Rabeprazole will lead to similar success.

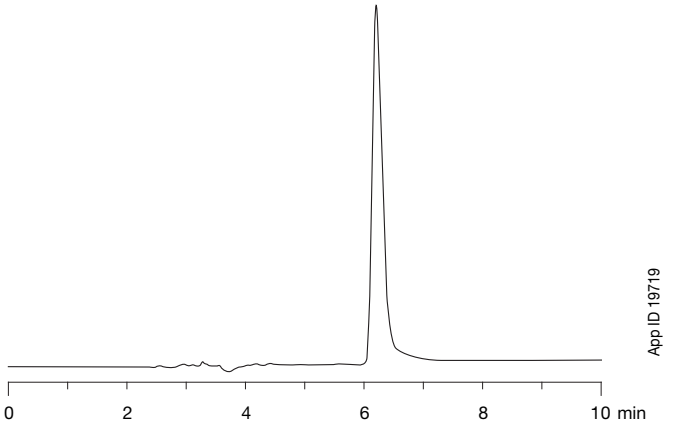
In this technical note, we described the successful separation under reversed phase conditions of both Pantoprazole and Rabeprazole. Based on previous work done at Phenomenex, the separation of Pantoprazole and Rabeprazole can be achieved without the use of base additives such as DEA[3]. Finally, these analytical reversed phase conditions can be developed and scaled-up for the preparative chiral purification of enantiomerically pure forms of racemic active ingredients Pantoprazole and Rabeprazole.

Fig. 2a Lansoprazole (Prevacid)



Column: Lux® 5 µm Cellulose-4
Dimensions: 250 x 4.6 mm
Part No.: 00G-4491-E0
Injection Volume: 1 µL
Concentration: 1 mg/mL
Mobile Phase: 90/10/0.1
Methanol/20 mMoL of NH_4HCO_3/DEA

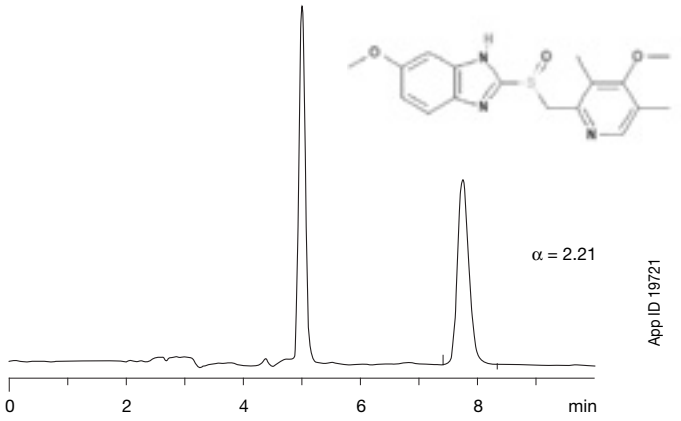
Fig. 2b Dexlansoprazole (Kapidex)



Column: Lux® 5 µm Cellulose-4
Dimensions: 250 x 4.6 mm
Part No.: 00G-4491-E0
Injection Volume: 0.15 µL
Concentration: 3.75 mg/mL
Mobile Phase: 90/10/0.1
Methanol/20 mMoL of NH_4HCO_3/DEA

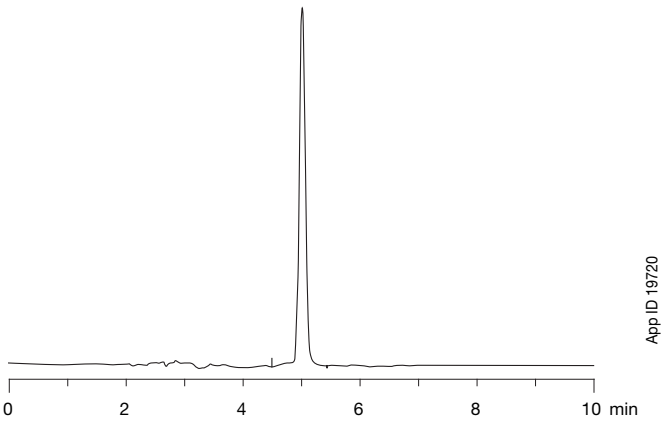
APPLICATIONS

Fig. 3a Omeprazole (Prilosec)



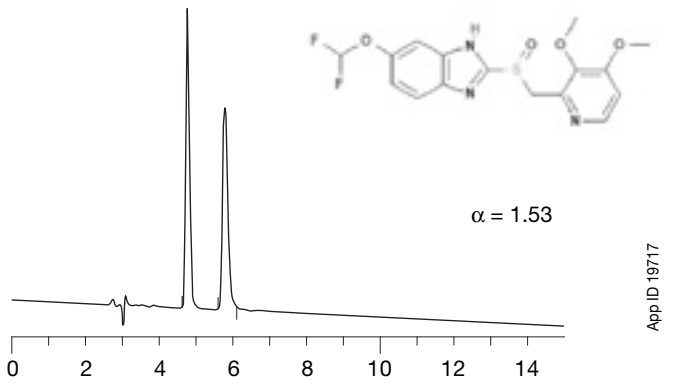
Column: Lux® 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm
Part No.: 00G-4457-E0
Injection Volume: 1 µL
Concentration: 1 mg/mL
Mobile Phase: 80/20/0.1
Acetonitrile/20 mMoL NH_4 HCO_3/DEA

Fig. 3b Esomeprazole (Nexium)



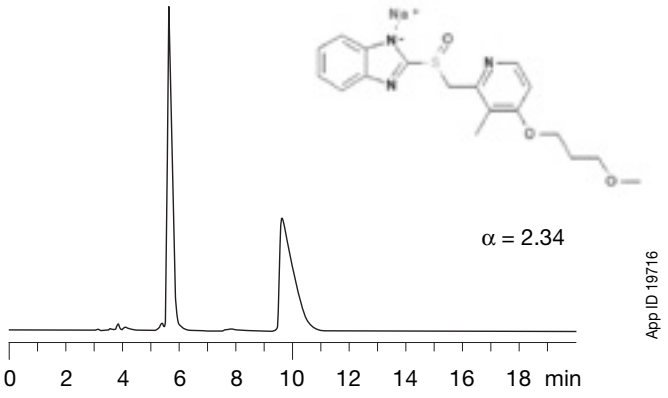
Column: Lux® 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm
Part No.: 00G-4457-E0
Injection Volume: 0.5 µL
Concentration: 2.5 mg/mL
Mobile Phase: 80/20/0.1
Acetonitrile/20 mMoL NH_4 HCO_3/DEA

Fig. 4 Pantoprazole (Protonic)



Column: Lux® 5 µm Cellulose-2
Dimensions: 250 x 4.6 mm
Part No.: 00G-4457-E0
Injection Volume: 10 µL
Concentration: 0.5 mg/mL
Mobile Phase: 60/40/10.1
Acetonitrile/10 mMoL NH_4 HCO_3/DEA

Fig. 5 Rabeprazole (Aciphex)



Column: Lux® 5 µm Cellulose-4
Dimensions: 250 x 4.6 mm
Part No.: 00G-4491-E0
Injection Volume: 10 µL
Concentration: 0.5 mg/mL
Mobile Phase: 80/20/0.1
Methanol/10 mMoL NH_4 HCO_3/DEA

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3. Phenomenex Technical Note TN-1079: Method Development for Reversed Phase Chiral LC/MS/MS Analysis of Stereoisomeric Pharmaceutical Compounds with Polysaccharide-Based Stationary Phases.

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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

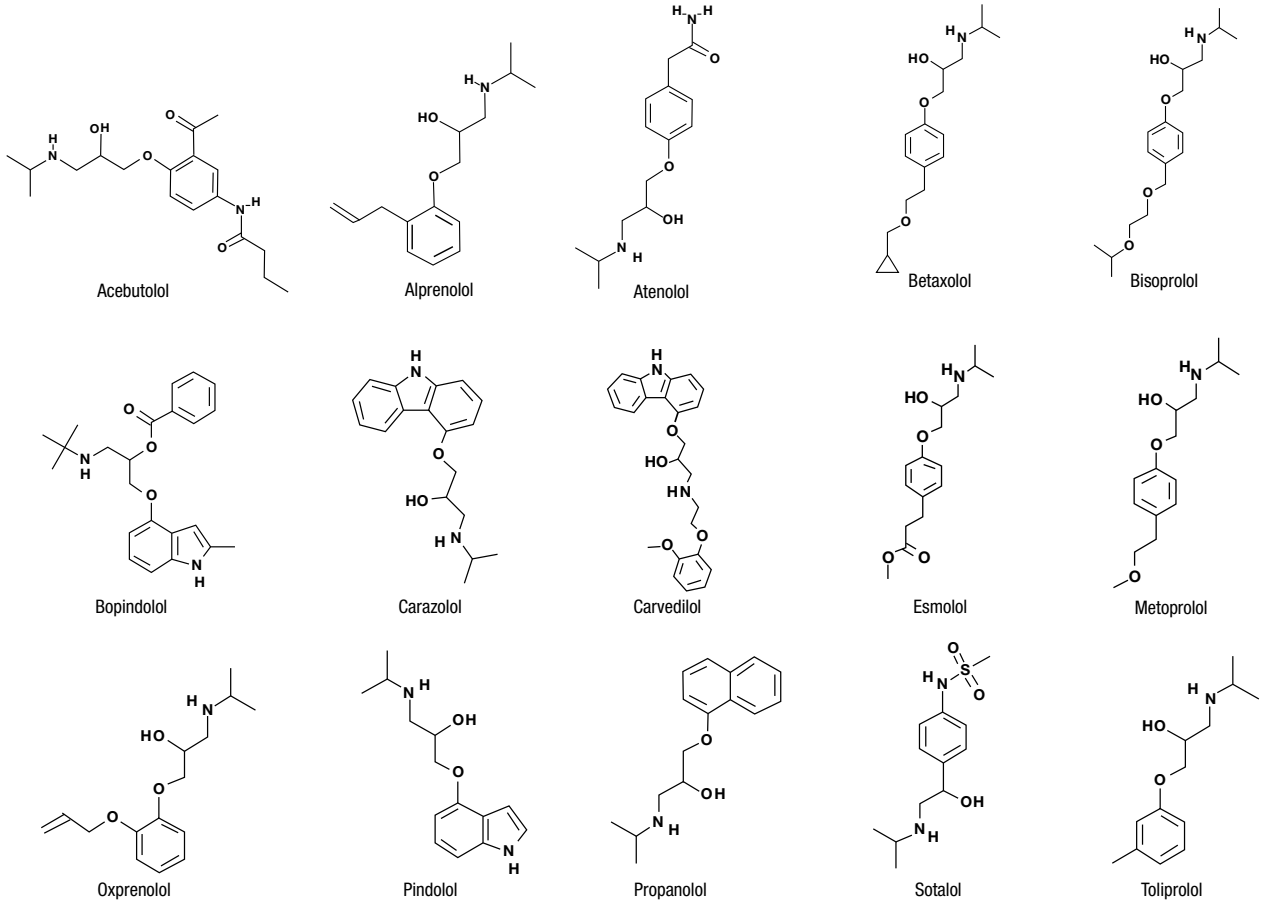
In this technical note, we report the chiral separation of various beta blocker pharmaceutical drugs using Lux polysaccharide-based chiral stationary phases. The reported separations are the results of a systematic screening of five different Lux phases in polar organic, normal phase, and reversed phase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



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

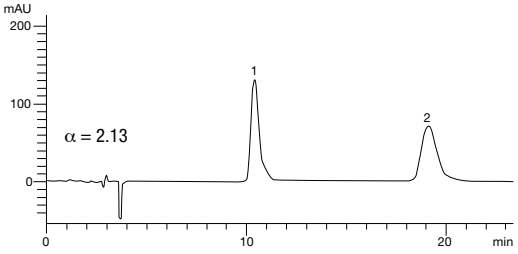
Beta Blocker	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Acebutolol	1978	Lux Amylose-2	4.21	4.91	PO	ACN/IPA (95:5) DEA (0.1 %)	18130
Alprenolol	2119	Lux Cellulose-2	1.12	6.12	NP	Hex/EtOH (95:5) DEA (0.1 %)	20443
Atenolol	2249	Lux Cellulose-1	1.39	10.55	NP	Hex/EtOH (80:20) DEA (0.1 %)	20547
Betaxolol	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.

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**.

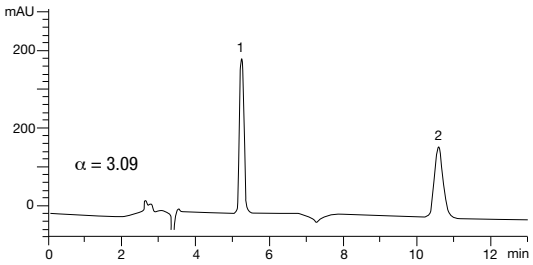
Figure 2.
Representative chromatograms for the separation of Beta Blockers

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.

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

Marc Jacob, Liming Peng, Michael Klein and Tivadar Farkas

Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

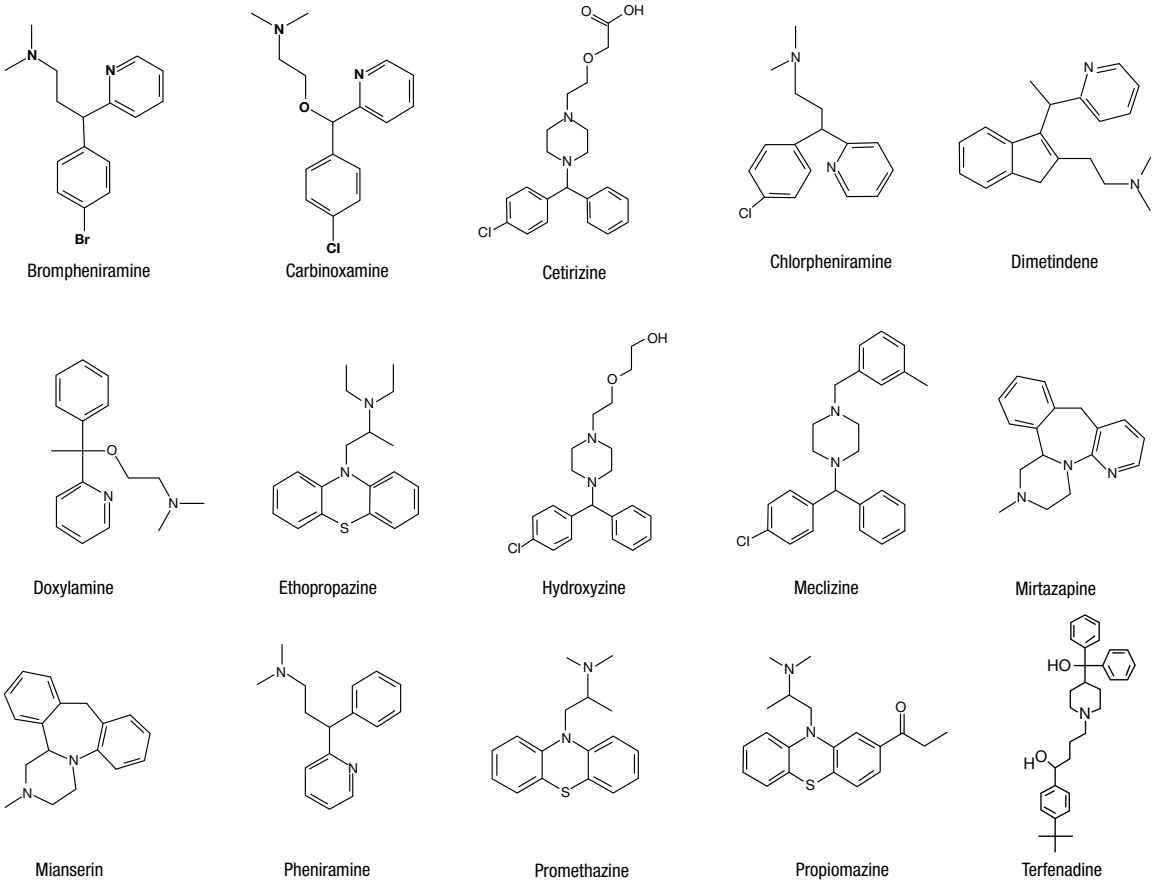
In this technical note, we report the chiral chromatographic separation of various anti-allergic drugs using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase, polar organic, and reversed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

Anti-allergic drugs, also known as histamine antagonists, are effective in the treatment of allergic reactions such as seasonal rhinitis and allergic dermatitis. The various anti-allergics 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 anti-allergic drugs racemic mixtures



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

Fifteen anti-allergic 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 anti-allergic drugs separated in this study are listed in **Table 1**. For each anti-allergic 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 anti-allergic drugs 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 10 min and all the separations are completed in less than 30 min. With basic analytes such as anti-allergics, 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 15 separations, 8 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.

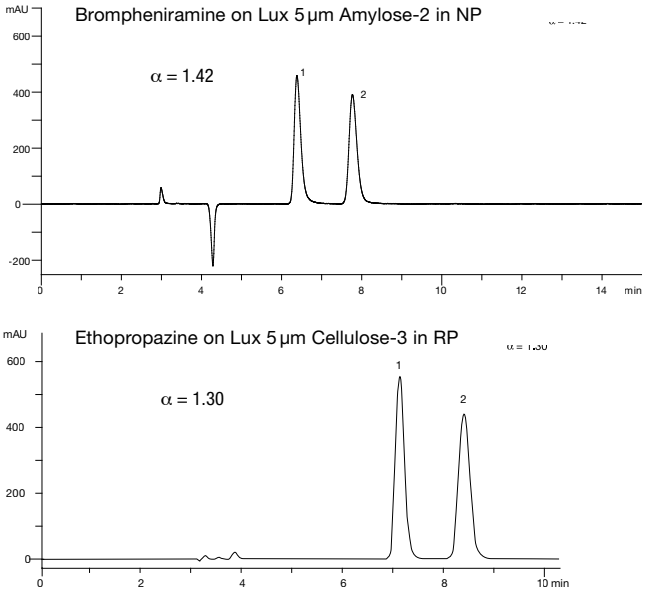
Table 1. Chiral separations of anti-allergic drugs using Lux polysaccharide-based CSPs

Beta Blocker	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Brompheniramine	6834	Lux Amylose-2	1.42	6.30	NP	Hex/IPA (90:10) DEA (0.1 %)	20082
Carbinoxamine	2564	Lux Amylose-2	1.28	6.69	NP	Hex/EtOH (90:10) DEA (0.1 %)	20452
Cetirizine	55182	Lux Cellulose-3	1.29	8.06	RP	ACN/20 mm NH_4HCO_3 (50:50) DEA (0.1 %)	19641
Chlorpheniramine	2725	Lux Amylose-2	1.98	6.94	NP	Hex/EtOH (95:5) DEA (0.1 %)	20445
Dimetindene	21855	Lux Cellulose-1	1.25	7.07	NP	Hex/EtOH (98:2) DEA (0.1 %)	20435
Doxylamine	3162	Lux Cellulose-4	1.91	6.04	NP	Hex/IPA (90:10) DEA (0.1 %)	20346
Ethopropazine	3290	Lux Cellulose-3	1.30	7.14	RP	MeOH/20 mm NH_4HCO_3 (95:5) DEA (0.1 %)	20303
Hydroxyzine	3658	Lux Cellulose-3	1.66	7.54	RP	MeOH/20 mm NH_4HCO_3 (80:20) DEA (0.1 %)	20320
Meclizine	4034	Lux Cellulose-3	2.62	5.52	NP	Hexane / EtOH (80:20) DEA (0.1 %)	20338
Mianserin	4184	Lux Cellulose-1	1.25	8.14	RP	MeOH/20 mm NH4HCO3 (90:10) DEA (0.1 %)	20225
Mirtazapine	4205	Lux Cellulose-2	1.32	5.80	PO	ACN/IPA (95:5) DEA (0.1 %)	20067
Pheniramine	4761	Lux Cellulose-3	1.17	5.47	NP	Hexane/EtOH (95:5) DEA (0.1 %)	20429
Promethazine	4927	Lux Cellulose-3	1.34	9.01	RP	MeOH/20 mm NH_4HCO_3 (95:5) DEA (0.1 %)	20306
Propiomazine	4940	Lux Cellulose-3	1.37	5.18	PO	MeOH/IPA (90:10) DEA (0.1 %)	20556
Terfenadine	5405	Lux Cellulose-2	1.30	7.00	NP	Hex/IPA (60:40) DEA (0.1 %)	20078

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.

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 Brompheniramine and Ethopropazine are shown in **Figure 2**.

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



Conclusion

In this study, we described the chiral separation of a variety of anti-allergic 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 10 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.

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4. Hamman, C.; Schmidt Jr., D. E.; Wong, M.; Hayes, M. J. *Chromatogr*. A **2011**, 1218, 7886– 7894.
5. Miller L. *J. Chromatogr*. A **2012**, 1250, 250. (Review).

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APPLICATIONS

Chromatographic Enantioseparation of Racemic Pain Relievers using Lux® Polysaccharide-Based Chiral Stationary Phases

Marc Jacob, Liming Peng, Michael Klein and Tivadar Farkas

Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

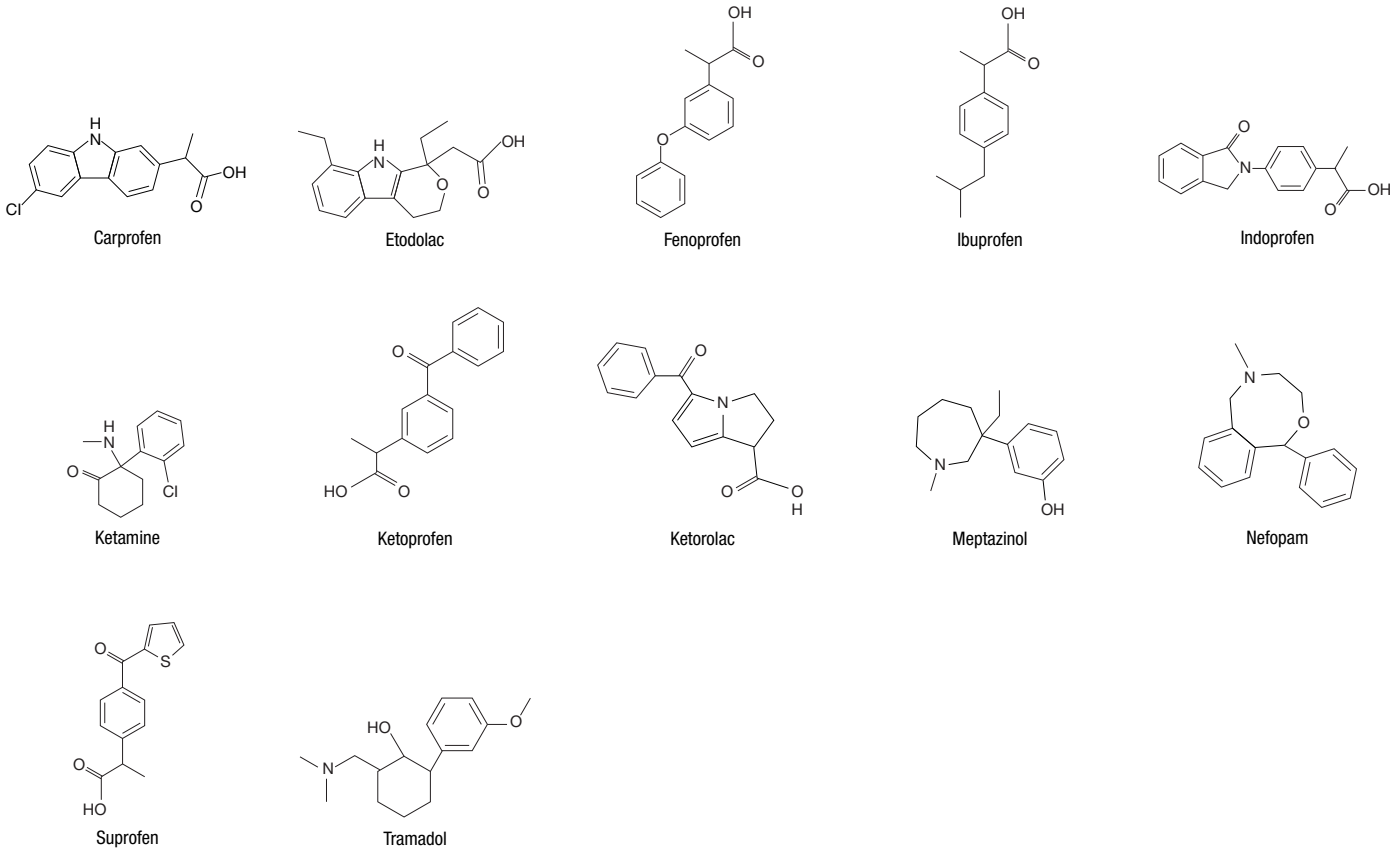
In this technical note, we report the chiral chromatographic separation of various anti-inflammatory agents and pain relievers using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in polar organic, 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]

Anti-inflammatory and analgesic agents are effective in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation. The pain relievers 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 structures of pain relievers and anti-inflammatory agents



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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

Pain reliever agents 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 pain relievers analyzed 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 anti-inflammatory agents and pain relievers 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 14 min and all the separations are completed in less than 30 min. With basic pain relievers, 0.1 % of diethylamine (DEA) was used as an additive, whereas with acidic derivatives, 0.1 % of formic acid (FA) was used as additive. Interestingly with pain reliever drugs, Lux Cellulose-3 phase was quite successful. Out of the 12 separations, 9 were most successful with Lux Cellulose-3.

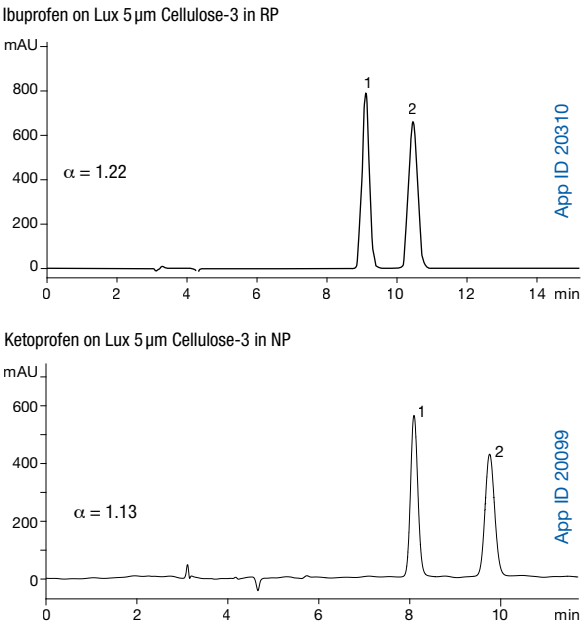
All of our Lux® products are pressure stable up to 300 bar and compatible with SFC separation mode[4] using an organic modifier such as MeOH, EtOH, IPA, or ACN. Two examples of chiral separation for Ibuprofen and Ketoprofen are shown in **Figure 2**.

Table 1. Chiral separations of anti-inflammatory agents using Lux polysaccharide-based CSPs

Compound	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Carprofen	2581	Lux Cellulose-3	1.20	7.69 min	NP	Hex/EtOH (60:40) FA (0.1 %)	20385
Etodolac	3308	Lux Cellulose-3	1.21	7.38 min	RP	ACN/FA (0.1 %) (40:60)	20324
Fenoprofen	3342	Lux Amylose-2	1.57	8.06 min	NP	Hexane/EtOH (95:5) FA (0.1 %)	20453
Ibuprofen	3672	Lux Cellulose-3	1.22	9.1 min	RP	MeOH/FA (0.1 %) (80:20)	20310
Indoprofen	3718	Lux Cellulose-3	1.17	12.29 min	RP	MeOH/FA (0.1 %) (80:20)	20296
Ketamine	3821	Lux Cellulose-3	2.42	5.49 min	RP	MeOH/20 mM NH_4HCO_3 (90:10) DEA (0.1 %)	20287
Ketoprofen	3825	Lux Cellulose-3	1.13	8.09 min	NP	Hex/IPA (80:20) FA (0.1 %)	20099
Ketorolac	3826	Lux Cellulose-3	1.52	8.65 min	PO	MeOH/IPA (90:10) FA (0.1 %)	20367
Meptazinol	41049	Lux Cellulose-3	1.38	7.74 min	NP	Hex/IPA (90:10) DEA (0.1 %)	20392
Nefopam	4450	Lux Cellulose-4	1.64	13.31 min	PO	MeOH/IPA (90:10) DEA (0.1 %)	20376
Suprofen	5359	Lux Cellulose-3	1.31	7.06 min	NP	Hex/EtOH (60:40) FA (0.1 %)	20098
Tramadol	33741	Lux Cellulose-1	1.13	5.86 min	RP	ACN/20 mM NH_4HCO_3 (50:50) DEA (0.1 %)	20240

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

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



Conclusion

In this study, we described the chiral separation of a variety of anti-inflammatory agents 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. 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. (Review)
4. Miller L. J. *Chromatogr. A* **2012**, *1250*, 250. (Review).

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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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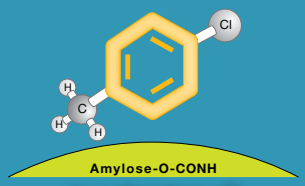

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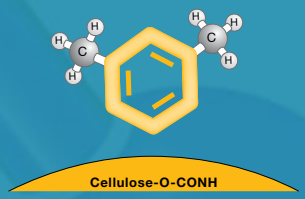
Amylose tris(5-chloro-2-methylphenylcarbamate)



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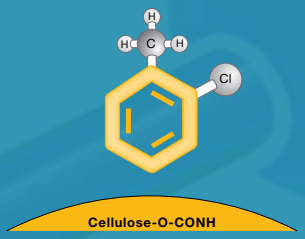
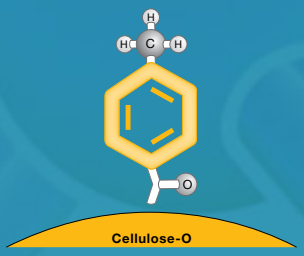
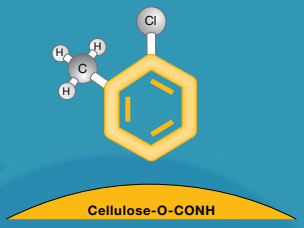
Cellulose tris(3, 5-dimethylphenylcarbamate)



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)

TN-1145

APPLICATIONS

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

Marc Jacob, Liming Peng, Michael Klein, Tom Cleveland and Tivadar Farkas

Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

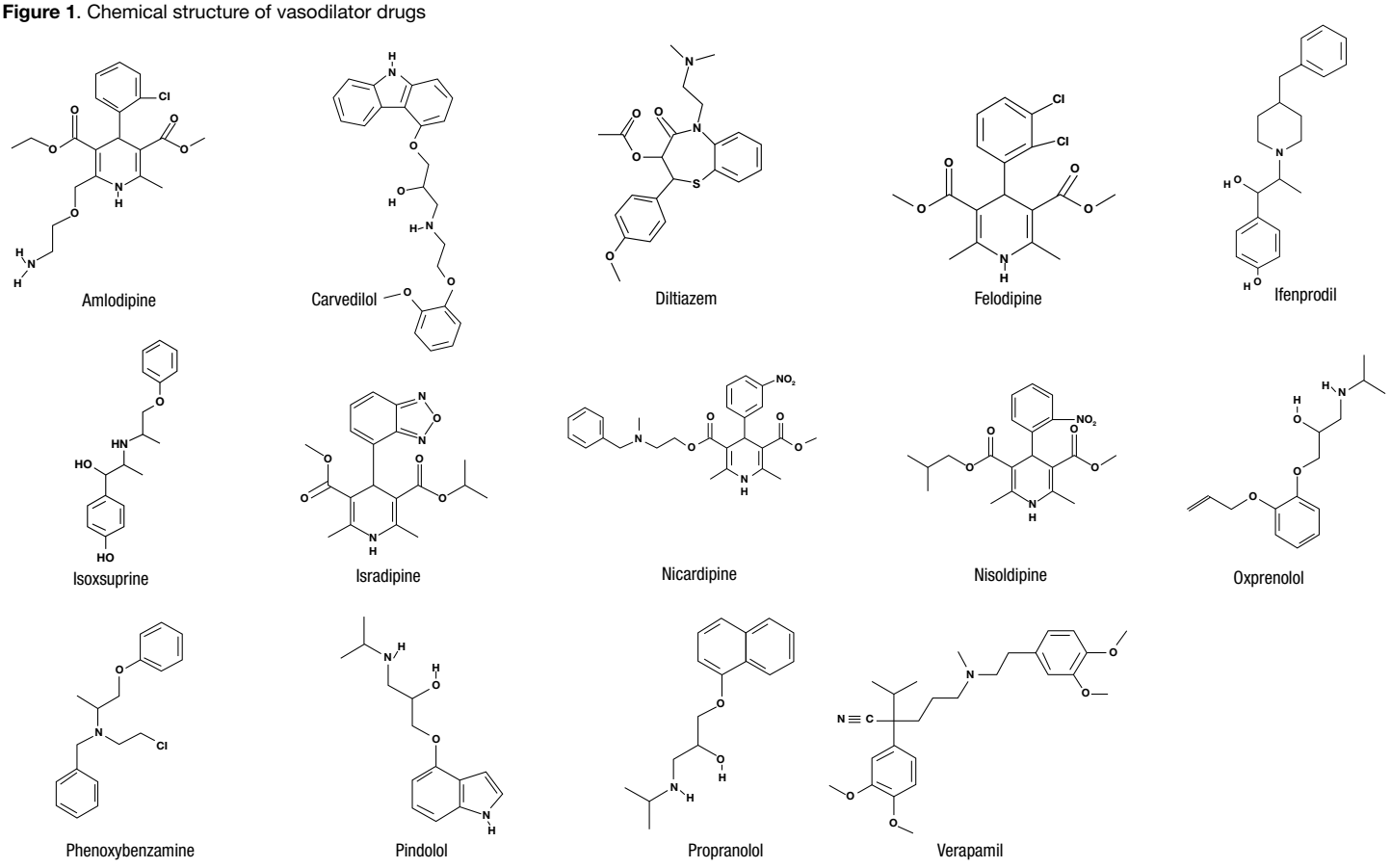
In this technical note, we report the chiral chromatographic separation of various vasodilator drugs using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase, polar organic, and reversed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

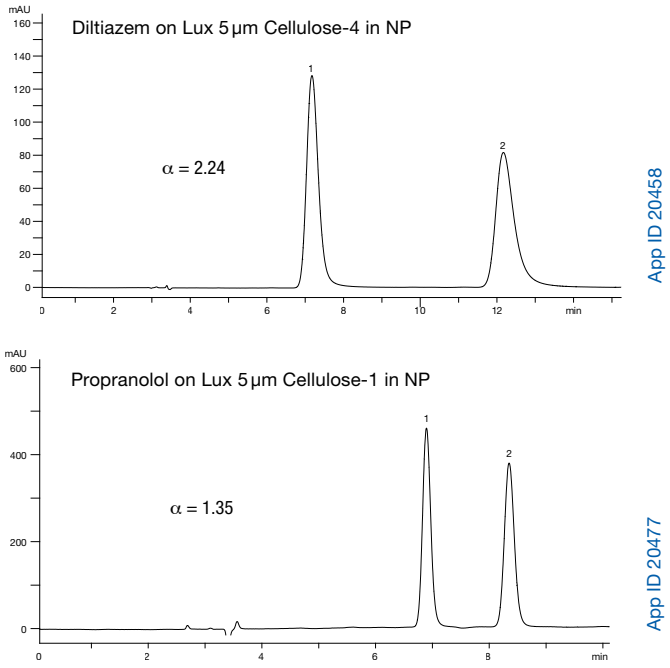
Compound	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Amlodipine	2162	Lux Cellulose-4	1.78	5.83 min	PO	ACN/IPA (95:5) DEA (0.1%)	20358
Carvedilol	2585	Lux Cellulose-4	1.74	6.79 min	NP	Hex/IPA (40:60) DEA (0.1%)	20422
Diltiazem	39186	Lux Cellulose-4	2.24	7.17 min	NP	Hex/IPA (60:40) DEA (0.1%)	20458
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

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 Diltiazem and Propanolol are shown in **Figure 2**.

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



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

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.
4. Hamman, C.; Schmidt Jr., D. E.; Wong, M.; Hayes, M. J. *Chromatogr*. A **2011**, 1218, 7886– 7894.
5. Miller L. *J. Chromatogr*. A **2012**, 1250, 250. (Review).

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

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

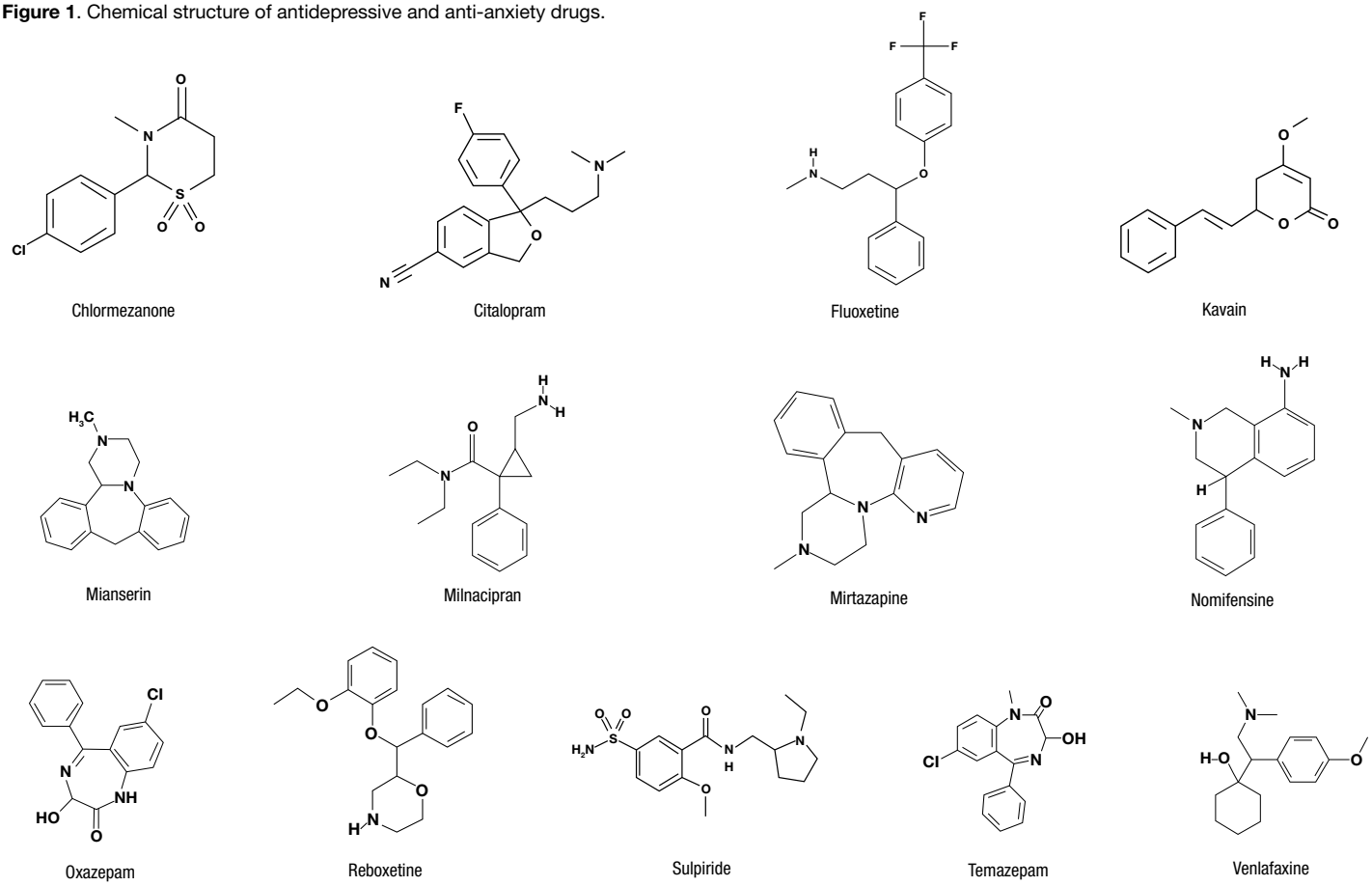
In this technical note, we report the chiral chromatographic separation of various antidepressive and anti-anxiety drugs using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase, polar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

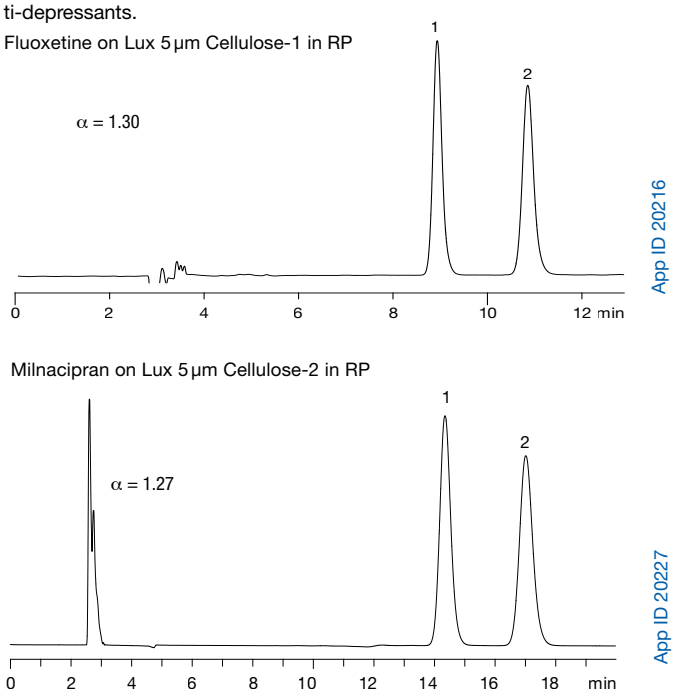
Table 1. Chiral separations of antidepressive and anti-anxiety drugs using Lux polysaccharide-based CSPs

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_4HCO_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_4HCO_3 (90:10) DEA (0.1 %)	20225
Milnacipran	65833	Lux Cellulose-2	1.27	11.46 min	RP	MeOH/20 mM NH_4HCO_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_4HCO_3 (90:10) DEA (0.1 %)	20329
Oxazepam	4616	Lux Cellulose-1	2.32	5.94 min	RP	ACN/20 mM NH_4HCO_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_4HCO_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.



Conclusion

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

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

Marc Jacob, Liming Peng, Michael Klein and Tivadar Farkas

Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

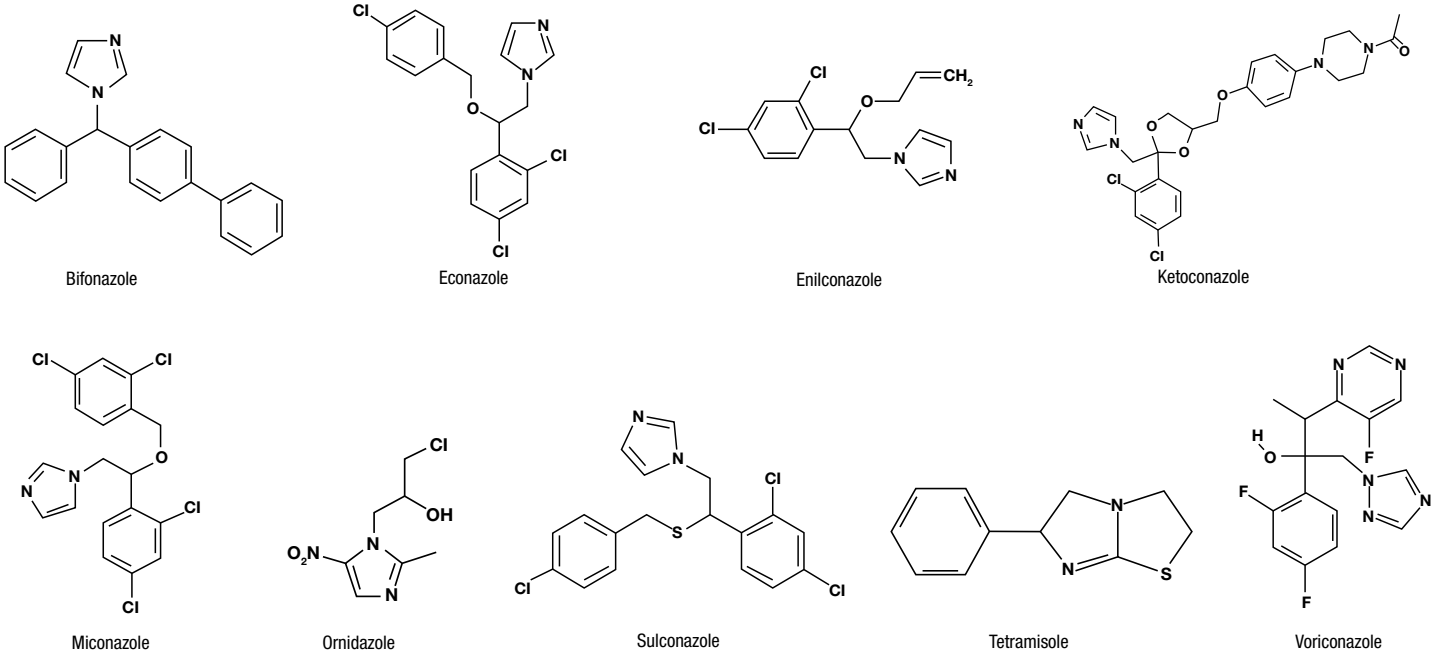
In this technical note, we report the chiral chromatographic separation of various antifungal agents using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase, polar organic, and reversed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

Imidazole and triazole antifungal drugs inhibit the enzyme responsible for converting lanosterol to ergosterol. Those drugs are effective in the treatment of fungal infections such as athlete's foot and ring worm. The various antifungal agents analyzed in this study are derived from imidazole or triazole and 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 antifungal agents.



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

Nine antifungal agents 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 antifungal agents separated in this study are listed in **Table 1**. For each antifungal agents 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 antifungal 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 14 min and all the separations are completed in less than 30 min. With basic analytes such as antifungal agents, 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 9 separations, 7 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 antifungal agents using Lux polysaccharide-based CSPs

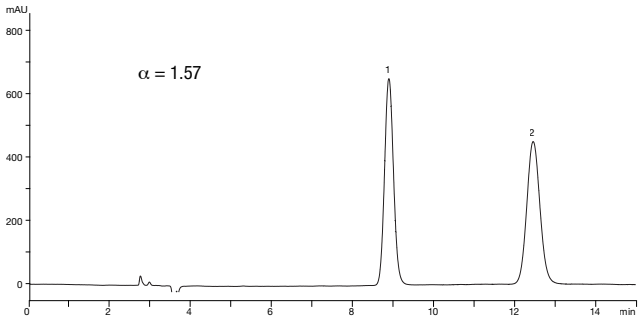
Compound	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Bifonazole	2378	Lux Cellulose-2	1.57	8.9 min	NP	Hex/EtOH (80:20) DEA (0.1 %)	20506
Econazole	3198	Lux Cellulose-3	2.84	5.92 min	NP	Hex/EtOH (40/:60) DEA (0.1 %)	20110
Enilconazole	37175	Lux Cellulose-4	1.39	7.19 min	NP	Hex/IPA (60:40) DEA (0.1 %)	20427
Ketoconazole	3823	Lux Cellulose-1	1.25	13.71 min	PO	MeOH/IPA (90:10) DEA (0.1 %)	20353
Miconazole	4189	Lux Cellulose-3	2.18	5.21 min	NP	Hex/EtOH (96:4) DEA (0.1 %)	20129
Ornidazole	28061	Lux Amylose-2	5.36	5.46 min	NP	Hex/IPA (40:60) DEA (0.1 %)	20530
Sulconazole	5318	Lux Cellulose-2	1.67	12.25 min	NP	Hex/IPA (40:60) DEA (0.1 %)	20126
Tetramisole	3913	Lux Cellulose-2	1.48	6.66 min	PO	ACN/IPA (95:5) DEA (0.1 %)	20284
Voriconazole	5231054	Lux Cellulose-4	4.16	7.26 min	NP	Hex/EtOH (20:80) DEA (0.1 %)	20421

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

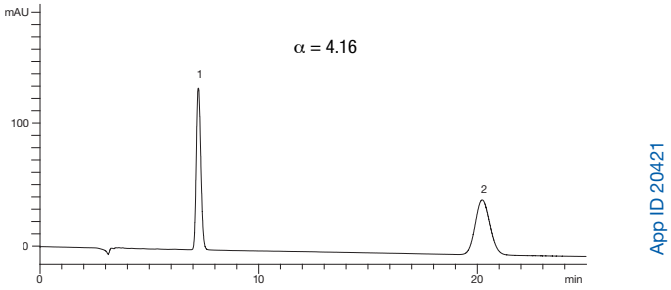
All of our Lux® products are pressure stable up to 300 bar and compatible with SFC separation mode using an organic modifier such as MeOH, EtOH, IPA, or ACN. Two examples of chiral separation for Bifonazole and Voriconazole are shown in **Figure 2**.

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

Bifonazole on Lux 5 µm Cellulose-2 in NP



Voriconazole on Lux 5 µm Cellulose-4 in NP



Conclusion

In this study, we described the chiral separation of a variety of antifungal agents 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 14 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.

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Clinical Drugs Applications

Chiral LC/MS/MS Method for Analyzing Metabolites of the Synthetic Cannabinoids JWH-018 and AM2201 Contained in K2/Spice Herbal Mixtures using Strata™-X-Drug B SPE and Lux® Cellulose-3 Chiral Column

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In this technote, we describe a new targeted metabolomic approach for assessing human synthetic cannabinoid exposure and pharmacology in blood and urine samples. The method utilizes a Solid Phase Extraction (SPE) step followed by chiral LC/MS/MS analysis using a Lux polysaccharide-based chiral stationary column providing a reliable and reproducible method that can be transferred to clinical research, forensic, and toxicology labs for analytical testing.

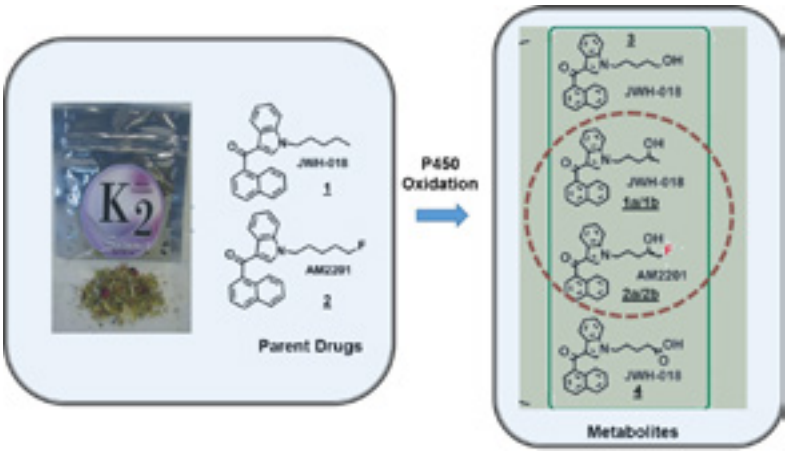
Introduction

Herbal mixtures labeled as "K2" or "Spice" are often marketed as legal marijuana substitutes to circumvent existing regulations and to avoid detection in standard drug screens. These products commonly contain the synthetic cannabinoid parent drugs JWH-018 (**Figure 1**, Parent Drug **1**) and AM2201 (**Figure 1**, Parent Drug **2**), both aminoalkylindoles and potent cannabinoid receptor agonists. With reports now indicating that 1 in 9 high school students experiment with synthetic cannabinoids and several medical reports specifically linking human injury and death to JWH-018 and AM2201, public health officials are increasingly concerned about abuse trends associated with these emerging cannabinoids.

Unfortunately, little is known about the metabolism and toxicology of these new drugs, but several clinical investigations identify the (ω -1)-hydroxyl metabolites enantiomers (**Figure 1**, Metabolites **1a**/**1b** or **2a**/**2b**), (ω)-hydroxyl (Metabolite **3**) and (ω)-carboxyl (Metabolite **4**) as primary biomarkers. These metabolites are also known to retain significant in vitro and in vivo pharmacological activity, which may offer a mechanistic explanation of the adverse effects associated with synthetic cannabinoid use. Since the (ω-1)-hydroxyl metabolites of JWH-018 and AM2201 are chiral molecules, analytical procedures capable of low level quantification of specific enantiomeric metabolites are required to further understand the metabolic and toxicological consequences of synthetic cannabinoid use.

This technote describes a novel LC/MS/MS method and SPE procedure capable of simultaneously resolving enantiomers as well as parent compounds and other related metabolites.

Figure 1.
Parent drugs and metabolic oxidation compound structures. The circled compounds are chiral metabolites.



Materials and Methods

Reagents and chemicals were obtained from Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Pittsburgh, PA) and Hemostat Laboratories (Dixon, CA). All sample and analytical standards including chiral isomers of JWH-018-(ω-1)-OH and AM2201-(ω-1)-OH were synthesized and provided by Cayman Chemical (Ann Arbor, MI). Strata-X-Drug B polymeric strong cation-exchange solid phase extraction cartridges, Lux Cellulose-3 analytical column and SecurityGuard™ were obtained from Phenomenex (Torrance, CA). Samples were prepared using a Gilson Nebula 215 solid phase extraction system (Middleton, WI) and analyzed using an Agilent® 1200 Series quaternary liquid chromatography system (Santa Clara, CA) interfaced with an API 4000™ QTRAP® tandem mass spectrometer (AB SCIEX, Framingham, MA). The operation of the HPLC system and mass spectrometer was controlled by Analyst® software (version 1.5.1, AB SCIEX, Framingham, MA).

Sample Pretreatment:

Urine sample

See Reference 1 for internal standard preparation and complete experimental details.

Blood sample

Pipette 50 µL of blood into 950 µL 0.1M sodium acetate buffer (pH 5.0) and spike with 10 µL of internal standard (IS) solution. The sample was then subjected to the SPE method described below.

SPE Procedure

Cartridge: Strata-X-Drug B, 30 mg/3 mL
Part No.: 8B-S128-TBJ
Condition: NOT REQUIRED
Equilibrate: NOT REQUIRED
Load: 1 mL pretreated sample
Wash: 1 mL Sodium acetate buffer
Wash: 1 mL Sodium acetate buffer/Acetonitrile (70:30)
Elute: 5 mL Ethyl acetate/Isopropanol (85:15)
Dry: Dry down completely under a stream of nitrogen @ 60 °C
Reconstitute: 100 µL Ethanol

HPLC Conditions

Column: Lux® 3 µm Cellulose-3
Dimensions: 150 x 2.0 mm
Part No.: 00F-4492-B0
Mobile Phase: A: 20 mM Ammonium bicarbonate
B: Acetonitrile

Gradient:	**Time (min)**	**B (%)**
	0	40
	10	95
	12	95
	15	40
	16	40

Flow Rate: 0.5 mL/min
Temperature: 40 °C
Detection: Tandem Mass Spectrometer (MS/MS)
Detector: API 4000™ QTRAP® (AB SCIEX)

Table 1.
Mass Spectrometry Parameters for Selective Reaction Monitoring (SRM)

Analyte	Q1 (m/z)	Q3 (m/z)
AM2201	360	155* 127†
(R)-(-)-AM2201-(ω-1)-OH	376	155* 127†
(S)-(+)-AM2201-(ω-1)-OH	376	155* 127†
JWH-018	342	155* 127†
JWH-018-(ω)-OH	358	155* 127†
JWH-018-(ω)-COOH	372	155* 127†
(R)-(-)-JWH-018-(ω-1)-OH	358	155* 127†
(S)-(+)-JWH-018-(ω-1)-OH	358	155* 127†

*Quantification Ion † Confirmation Ion

Results and Discussion

JWH-018 is metabolized in humans to form the (ω)-monohydroxylated, (ω)-carboxylated, and (ω-1)-monohydroxylated metabolites. AM2201 exposure leads to the formation of common (ω)-JWH-018 metabolites but also the distinct (ω-1)-monohydroxylated AM2201 metabolites (**Figure 1**). A targeted metabolomic approach that simultaneously measures each primary metabolite including the enantiomeric (ω-1)-metabolites is required to facilitate future clinical studies designed to understand the relationship between drug metabolism and clinical symptoms documented after JWH-018 and AM2201 use. This new chiral LC/MS/MS approach achieves this requirement by resolving all metabolites of interest, including the R and S enantiomers of the (ω-1)-monohydroxylated metabolites of JWH-018 and AM2201 in human urine and blood (**Figures 2** and **3**). The chromatography of standards, QC samples, and unknown urine specimens is similar for all matrices evaluated. Retention times established for each analyte internal standard remained constant (±0.1 min). All calibration curves were linear over the tested analytical range, where r^2 values were ≥0.99. The lower limits of quantification (LLOQ) for each analyte are comparable to previous LLOQ measurements reported with similar methods and mass spectra are consistent with reference libraries previously reported. [2,3]

Figures 2 and **3** represent LC/MS/MS chromatograms produced from 10 ng/mL and 5 ng/mL (respectively) synthetic cannabinoid quality control samples in human urine and blood (all synthetic cannabinoids standard were provided by Cayman Chemical). The chromatography was similar in all standards. The different color tracings are representative of the Specific Reaction Monitoring (SRM) experiments for each specific metabolite (see **Table 1** for SRM transitions).

Figure 2.
LC/MS/MS chromatograms produced from a representative 10 ng/mL quality control sample prepared in pooled human urine

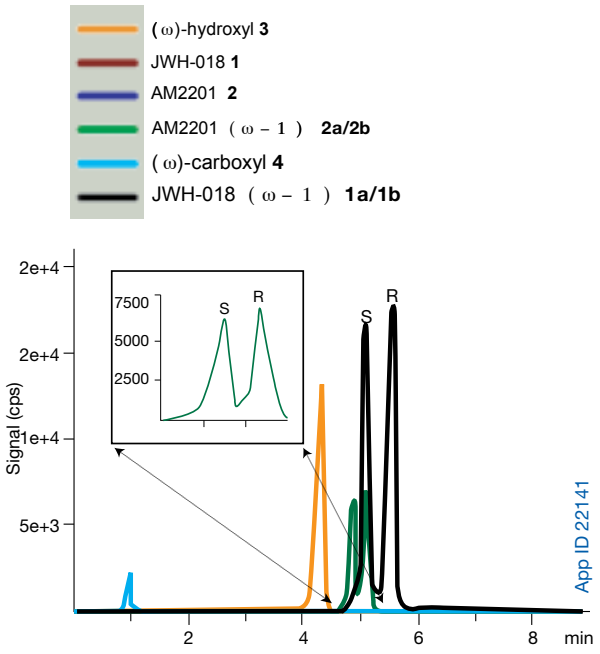
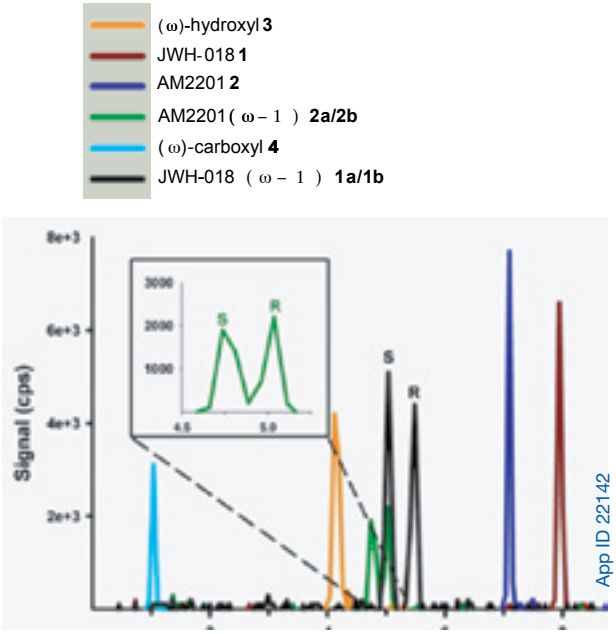


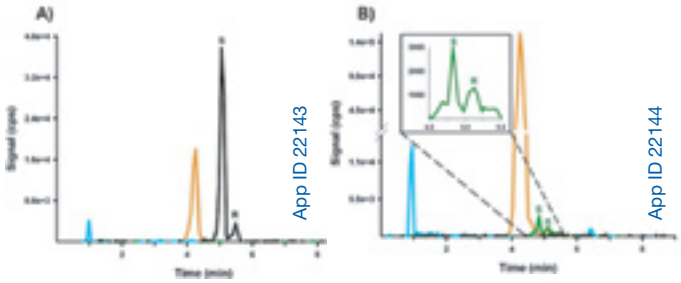
Figure 3.
LC/MS/MS chromatograms produced from a representative 5 ng/mL quality control sample prepared in blood



APPLICATIONS

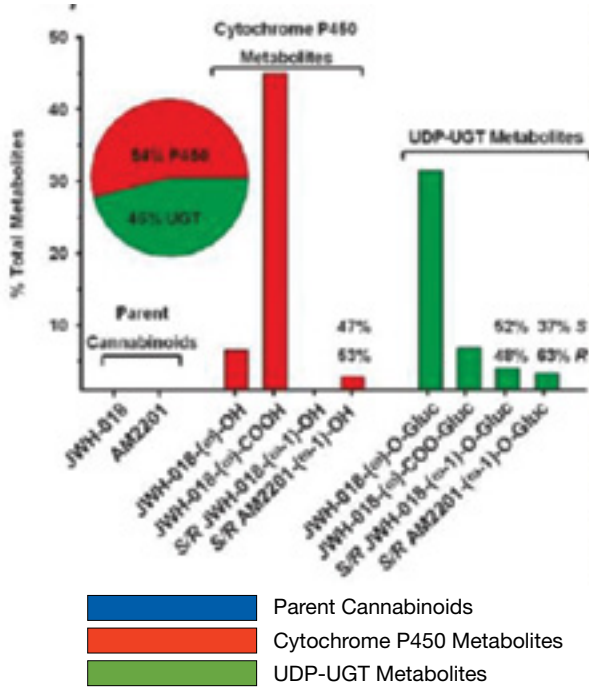
Figures 4a and **4b** show representative LC/MS/MS chromatograms of human samples which tested positive for the (ω-1)-monohydroxylated metabolite of JWH-018 (**Figure 1**, Metabolites **1a**/**1b**) and for the (ω-1)-monohydroxylated metabolite of AM2201 (**Figure 1**, Metabolites **2a**/**2b**). As shown in **Figure 1**, the (ω-1)-monohydroxylated metabolites are unique biomarkers for each respective synthetic cannabinoid.

Figure 4.
Representative LC/MS/MS chromatograms produced from (**A**) a human sample positive for the (ω-1)-monohydroxylated metabolite of JWH-018, and (**B**) a human urine sample positive for the (ω-1)-monohydroxylated metabolite of AM2201



In **Figure 5,** we demonstrate how this method was used to generate the metabolic profile of a human urine specimen which tested positive for JWH-018/AM2201 metabolites. The relative percentage of each metabolite is represented and the relative percentage of S or R enantiomers is provided above the bar of the corresponding (ω-1)-monohydroxylated metabolite.

Figure 5.
Metabolic profile generated from a human urine sample which tested positive forJWH-018/AM2201 metabolites



JWH-018 and AM2201 are both subjected to cytochrome-P450 mediated oxidation as well as uridine diphosphate glucuronyltransferase (UGT) conjugation during the metabolism process. Cytochrome-P450 metabolizes JWH-018 and AM2201 in the lung and liver while UGT is thought to be responsible for conjugating each metabolite with glucuronic acid. The pie chart inset compares the total relative percentage of free cytochrome P450 metabolites versus the total relative percentage of glucuronic acid conjugates. The conjugation percentage was determined by measuring metabolite concentrations pre- and post-ω-glucuronidase treatment (see Reference 1 for full details). These results show that when patients are exposed to only JWH-018 (**Figure 5**), the JWH-018 (ω-1)-monohydroxylated metabolite was excreted in a much higher concentration as compared to the other JWH-018 metabolites studied. In contrast, AM2201 (ω-1)-monohydroxylated enantiomers were not preferentially excreted. This indicates that UGTs may exhibit stereospecificity toward chiral synthetic cannabinoid metabolites.

Conclusion

The LC/MS/MS method described in this technical note is capable of fully resolving and quantifying chiral metabolites of JWH-018 and AM2201 as well as parent drugs. The precision and accuracy measurements are similar to previously developed clinical and forensic assays which make this method easily transferrable to clinical research, forensic, and toxicology labs for analytical testing. Moreover, this chiral method can help researchers in the understanding and evaluation of the clinical toxicity, pharmacodynamics and pharmacokinetics of achiral and chiral synthetic cannabinoid metabolites produced from JWH-018 and AM2201.

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3. Moran, J. H. *et al*. Anal. Chem. **2011**, 83, 4228– 4236.

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Pesticide Applications

Chromatographic Enantioseparation of Racemic Herbicide Agents using Lux® Polysaccharide-Based Chiral Stationary Phases

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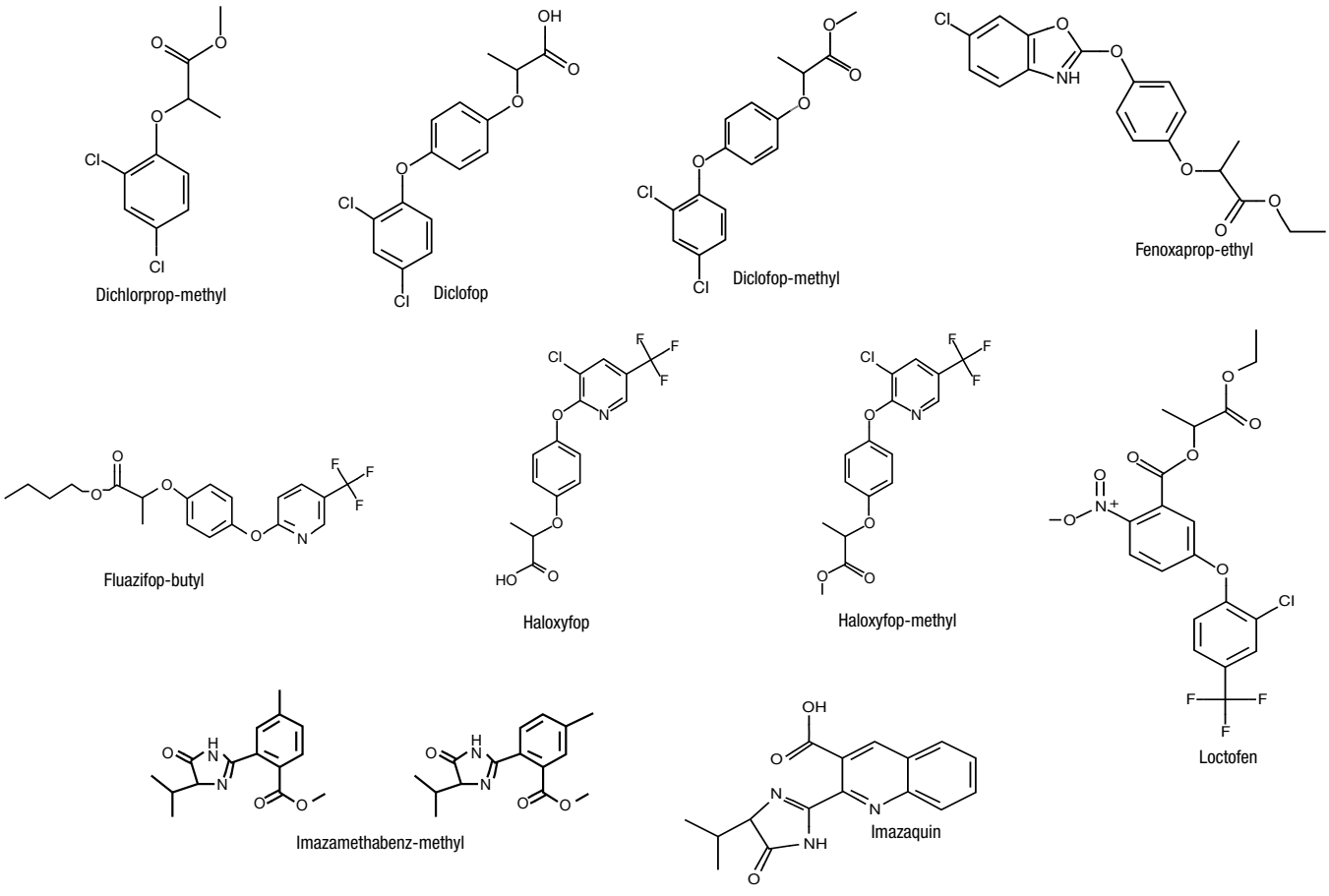
In this technical note, we report the chiral chromatographic separation of various herbicide agents using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase and reversed phase separation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

Introduction

Herbicides have many positive uses such as increasing food production, decreasing damage to crops, reducing plant diseases, and more, but they also pose risks to humans and the environment. Of the 1,693 pesticides listed in a recent review,[1] 482 (28 %) are chiral (chemical compounds containing one or more centers of asymmetry) of which 150 are classified as herbicides. The mode of action for many herbicides is to interfere with chiral plant hormones controlling growth and, therefore, the configuration of the herbicides plays a role in efficacy. As a result, some of those herbicides, such as dichlorprop-methyl, diclofop-methyl, fenoxaprop-ethyl, and haloxyfop-methyl are produced as single or enriched stereoisomer formulation. Additionally, the degradation of those chiral herbicides by soil microbes is enantioselective[2] and each enantiomer will be eliminated from the environment following a different pathway. The degradation difference of chiral herbicides, combined with possible enantiospecific toxicity can affect not only efficacy, but also exposure and risk to humans and environment. In the pharmaceutical industry, mainly due to the potential enantiospecific toxicity, chiral drugs are routinely tested for chiral purity, whereas pesticides generally are not.

Separations of chiral compounds can be performed by chiral chromatography using chiral stationary phases (CSPs) in high performance liquid chromatography (HPLC). HPLC is recognized as the most popular and reliable tool for both analytical and preparative separation of chiral compounds. As a matter of fact, 76 % of the analytical chiral separations reported in the recent chiral pesticides review[1] were performed by HPLC, and gas chromatography (GC) was second with 18 % of the separations reported. Polysaccharide-based CSPs such as Lux are the most widely used phases for the chromatographic separation of enantiomers.[3,4] Those CSPs show excellent success rate for chiral separation of a broad range of chiral compounds, as well as high loading ability for preparative applications. The various herbicide agents analyzed in this study are depicted in **Figure 1**.

Figure 1. Chemical structure of herbicides agents racemic mixtures separation modes



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

Eleven racemic herbicide agents 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) 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 herbicide agents 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 compounds of this type. All the herbicides 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 21 min. With basic and neutral herbicides derivatives, 0.1 % of diethylamine (DEA) was used as an additive whereas with acidic derivatives 0.1 % of formic acid (FA) was used as the additive. The presence of DEA favors dissociation of the amino group and improves peak shape. A similar effect is observed with formic acid as the additive with acidic pain reliever such as Dichlofop and Haloxyfop.

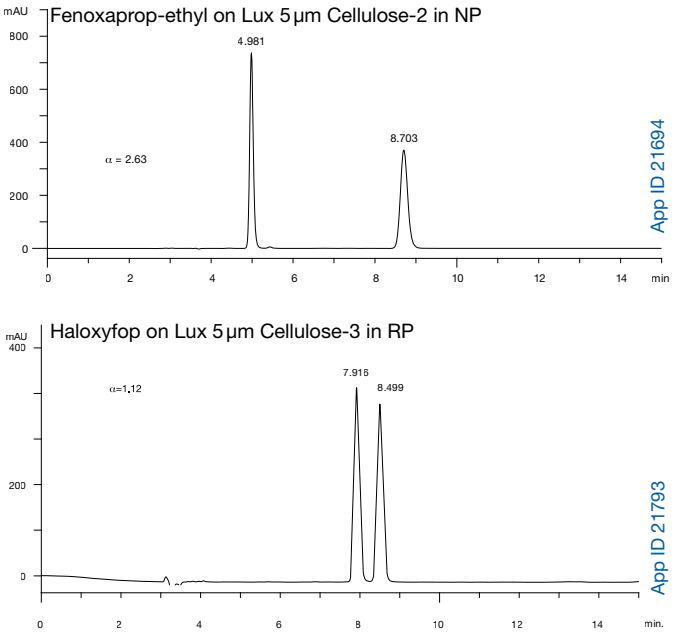
Table 1. Chiral separations of herbicides agents using Lux polysaccharide-based CSPs

Compound	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Dichlorprop-methyl	90988	Lux Amylose-2	1.06	19.22	RP	ACN/20 mM NH_4HCO_3 (40:60) DEA (0.1 %)	21761
Diclofop	38687	Lux Amylose-2	1.25	8.94	NP	Hex/IPA (80:20) FA (0.1 %)	21687
Diclofop-methyl	39985	Lux Cellulose-1	2.51	6.23	NP	Hex/IPA (80:20) DEA (0.1 %)	21688
Fenoxaprop-ethyl	47938	Lux Cellulose-2	2.63	4.98	NP	Hex/IPA (80:20) DEA (0.1 %)	21694
Fluazifop-butyl	50897	Lux Cellulose-3	1.31	7.89	RP	ACN/20 mM NH_4HCO_3 (60:40) DEA (0.1 %)	21786
Haloxyfop	50895	Lux Cellulose-3	1.12	7.92	RP	ACN / H_2O (50:50) FA (0.1 %)	21793
Haloxyfop-methyl	50896	Lux Amylose-2	1.21	6.34	NP	Hex/IPA (80:20) DEA (0.1 %)	21707
Imazamethabenz-methyl	54744	Lux Cellulose-4	1.24	7.75	NP	Hex/IPA (80:20) DEA (0.1 %)	21711
Imazaquin	54739	Lux Cellulose-3	1.38	5.06	NP	Hex/EtOH (60:40) DEA (0.1 %)	21714
Loctofen	62276	Lux Cellulose-2	1.37	7.11	NP	Hex/IPA (80:20) DEA (0.1 %)	21716

ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, H_2O = Water, FA = Formic acid, 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. Two examples of chiral separation for Fenoxaprop-ethyl and Haloxyfop are shown in **Figure 2**.

Figure 2.
Representative chromatograms for the chiral separation of herbicides.



Conclusion

In this study, we described the successful chiral separation of a variety of herbicide agents 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.

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Enantiomeric and Diastereoisomeric Resolutions of Chiral Triazole Fungicides using Lux® Polysaccharide-Based Chiral Stationary Phases

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Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

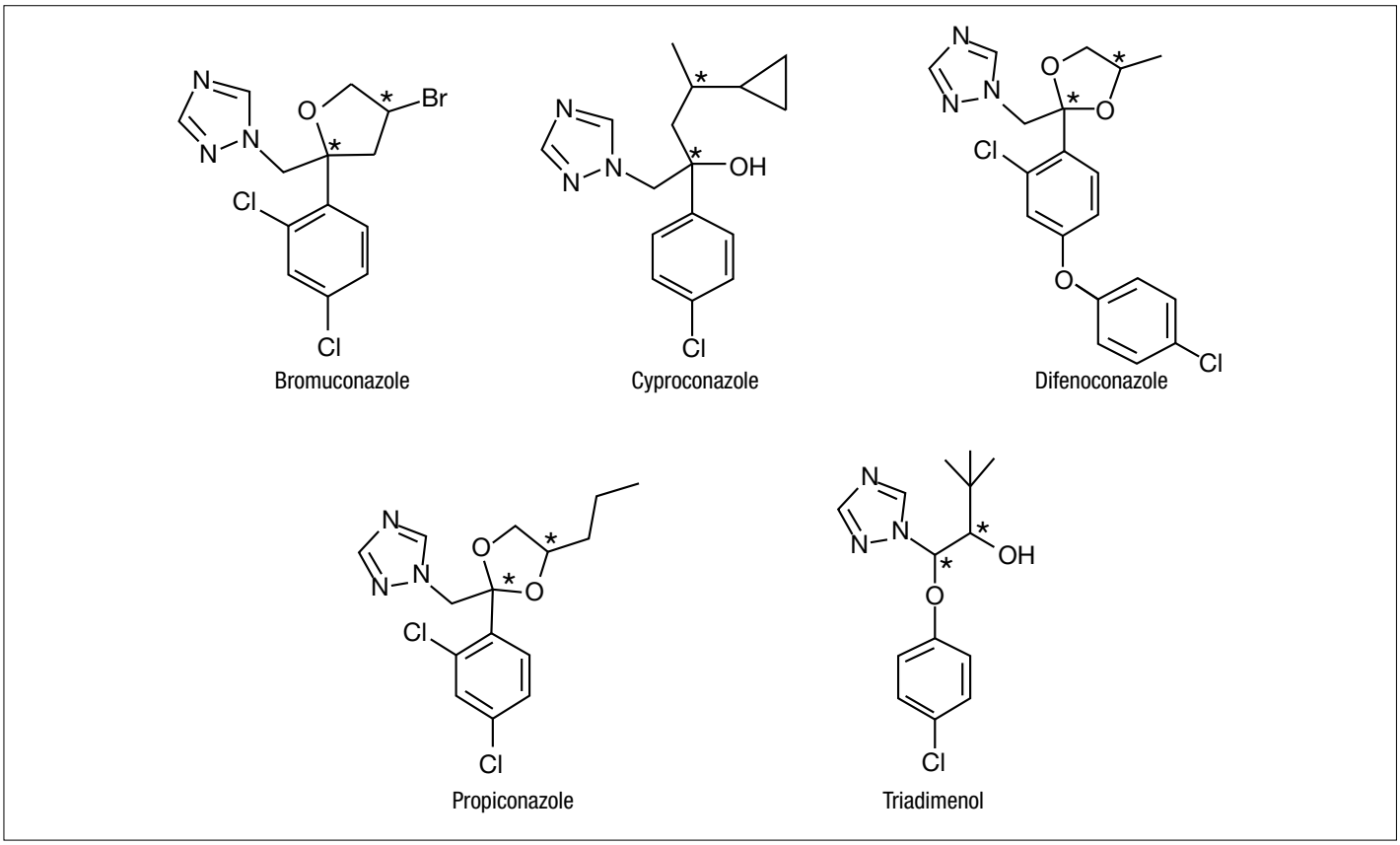
In this technical note, we report the enantiomeric and diastereoisomeric separations of five fungicides containing two stereogenic centers using Lux polysaccharide-based chiral stationary phases. The reported separations are the results of a systematic screening of five different Lux phases in normal phase and reversed phase separation modes. For each compound screened, baseline resolution of the four different stereoisomers is provided with a run time below 25 minutes.

Introduction

Fungicides have many positive uses such as increasing food production, decreasing damage to crops, reducing plant diseases, and more, but they also pose risks to humans and the environment. Of the 1693 pesticides listed in a recent review,[1] 482 (28 %) are chiral (chemical compounds containing one or more centers of asymmetry) of which 104 are classified as fungicides. The degradation of those chiral fungicides by soil microbes is stereoselective and each stereoisomer will be eliminated from the environment following a different pathway.[2,3] The degradation difference of chiral fungicides, combined with possible stereospecific toxicity can affect not only efficacy, but also exposure and risk to humans and environment.[3] In the pharmaceutical industry, mainly due to the potential stereospecific toxicity, chiral drugs are routinely tested for chiral purity, whereas pesticides generally are not.

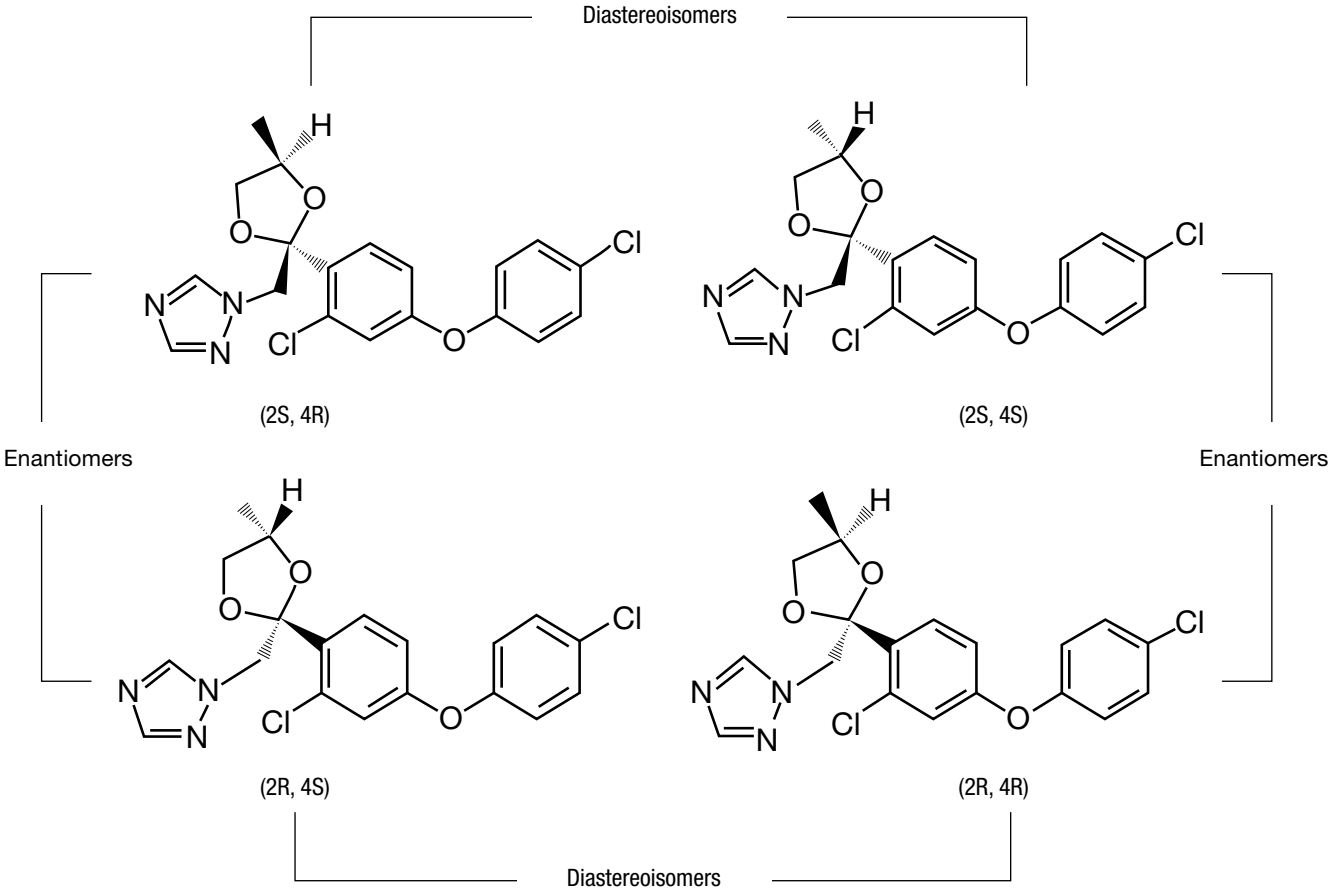
In this application note, we present the enantiomeric and diastereoisomeric separations of five triazole fungicides: Bromuconazole, Cyproconazole, Difenoconazole, Propiconazole, and Triadimenol. The chemical structure for each fungicide is represented in **Figure 1**.

Figure 1.
Chemical structure of chiral fungicides



All triazole fungicides evaluated in this application contain two stereogenic centers and therefore can have four stereoisomers as depicted in **Figure 2** for the example of Difenoconazole. The stereoisomers that are mirror images are also called enantiomers (SS/RR and SR/RS). Enantiomers can be separated from each other by chiral chromatography using chiral stationary phases (CSPs) in high performance liquid chromatography (HPLC). HPLC is recognized as the most popular and reliable tool for both analytical and preparative separation of chiral compounds.[4] As a matter of fact, 76 % of the analytical chiral separations reported in the recent chiral pesticides review[1] were performed by HPLC; gas chromatography (GC) was second with 18 % of the separations reported. Polysaccharide-based CSPs such as Lux® are the most widely used phases for the chromatographic separation of enantiomers.[4,5] Those CSPs show excellent success rate for chiral separation of a broad range of chiral compounds, as well as high loading ability for preparative applications under both, HPLC[6] and supercritical fluid chromatography (SFC)[7].

Figure 2.
Structure of stereoisomeres for Difenoconazole



Material and Methods

All HPLC 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

The five triazole fungicides depicted in **Figure 1** were analyzed on Lux polysaccharide-based CSPs (Cellulose-1, Cellulose-2, Cellulose-3, Cellulose-4, and Amylose-2) in normal phase (NP) and reversed phase (RP) separation modes. After performing a systematic screening, the separations that showed optimum resolution between all the peak were selected, even though in most of the cases, alternative separation was obtained with other Lux phases and/or modes. The separation results as well as the selectivity between each stereoisomer are summarized in **Table 1**. For each fungicide screened, we provide the chemical identification number (CID). This unique number can be linked to The PubChem Project website for further research regarding each compound's pharmaceutical properties. Additionally, the Lux phases used, the retention time of the first and last stereoisomers, as well as the isocratic conditions used for each compound are listed in **Table 1**. As expected, polysaccharide-based Lux columns are quite successful at resolving chiral compounds of this type. For each fungicide tested, all the stereoisomers are separated with selectivity greater or equal to 1.1 between adjacent peaks. In the last column of the **Table 1**, the corresponding Phenomenex application number is provided. Those applications are easily accessible on our website (www.phenomenex.com) and can be searched by application number, structure, CID, or compound name.

Table 1.
Enantiomeric and diastereoisomeric separations of fungicides using Lux polysaccharide-based CSPs

Analyte	CID	CSPs	Mobile Phase	Rt_1 (min)	Rt_4 (min)	$\alpha_{(1,2)}$	$\alpha_{(2,3)}$	$\alpha_{(3,4)}$	App ID
Bromuconazole	3444	Lux Cellulose-2	ACN/20 mM NH_4HCO_3 (60:40) DEA (0.1 %)	14.63	23.18	1.36	1.14	1.11	21751
Cyproconazole	86132	Lux Cellulose-4	ACN/20 mM NH_4HCO_3 (60:40) DEA (0.1 %)	6.44	9.16	1.20	1.18	1.25	21755
Difenoconazole	86173	Lux Cellulose-3	Hexane/EtOH (85:15) DEA (0.1 %)	11.07	15.10	1.07	1.07	1.29	21681
Propiconazole	43234	Lux Cellulose-1	Hexane/IPA (80:20) DEA (0.1 %)	6.83	10.16	1.33	1.21	1.12	21726
Triadimenol	41368	Lux Cellulose-2	Hexane/IPA (80:20) DEA (0.1 %)	5.06	7.58	1.28	1.36	1.19	21739

ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, DEA = Diethylamine, NH_4HCO_3 = Ammonium bicarbonate

The enantiomeric and diastereoisomeric separations for the stereoisomers of Bromuconazole, Cyproconazole, Difenoconazole, Propiconazole and Triadimenol are respectively shown in **Figure 3, 4, 5, 6** and **7**.

Figure 3.
Stereoselective HPLC analysis on the stereoisomers mix of Bromuconazole

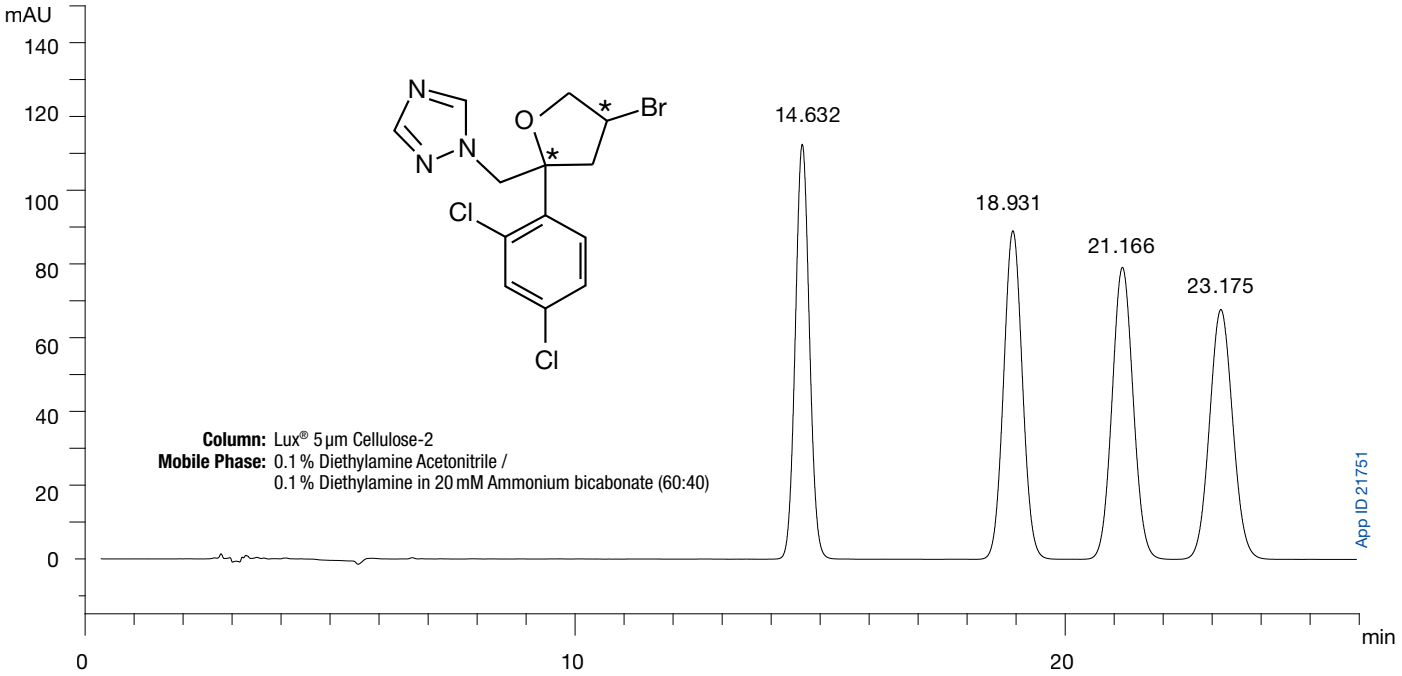


Figure 4.
Stereoselective HPLC analysis on the stereoisomers mix of Cyproconazole

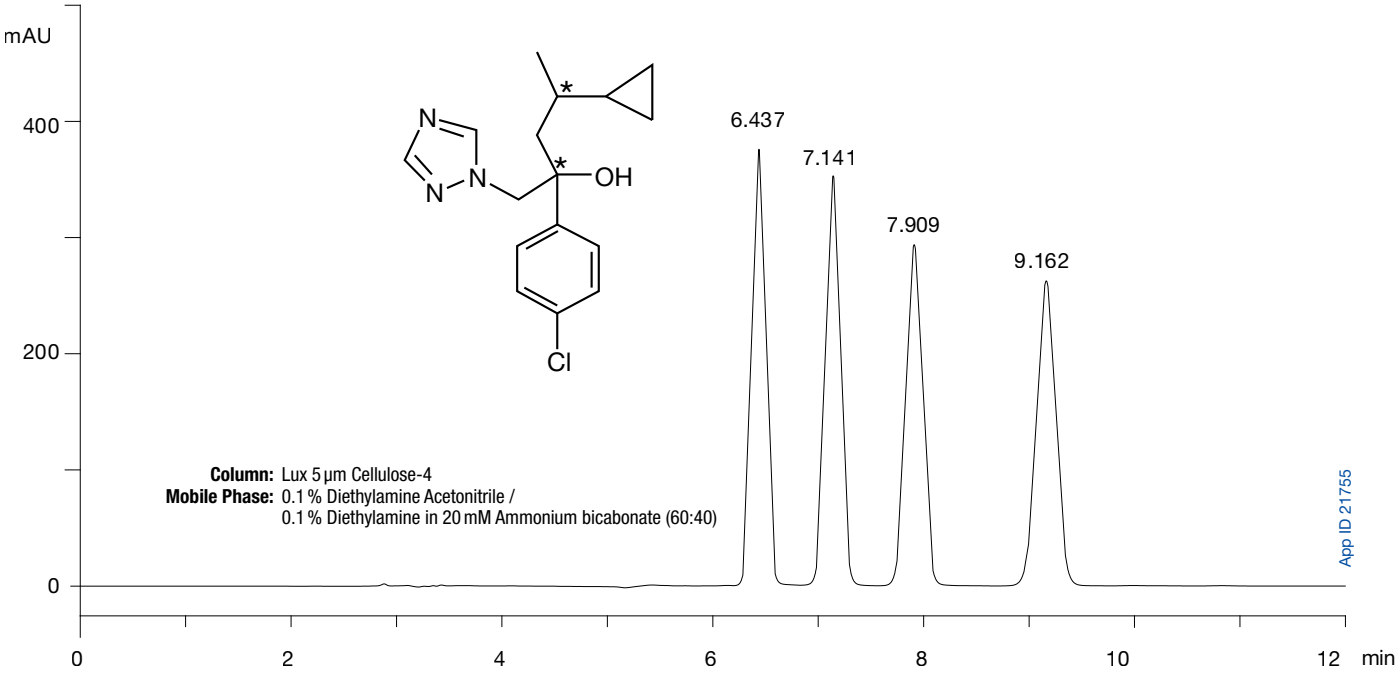


Figure 5.
Stereoselective HPLC analysis on the stereoisomers mix of Difenoconazole

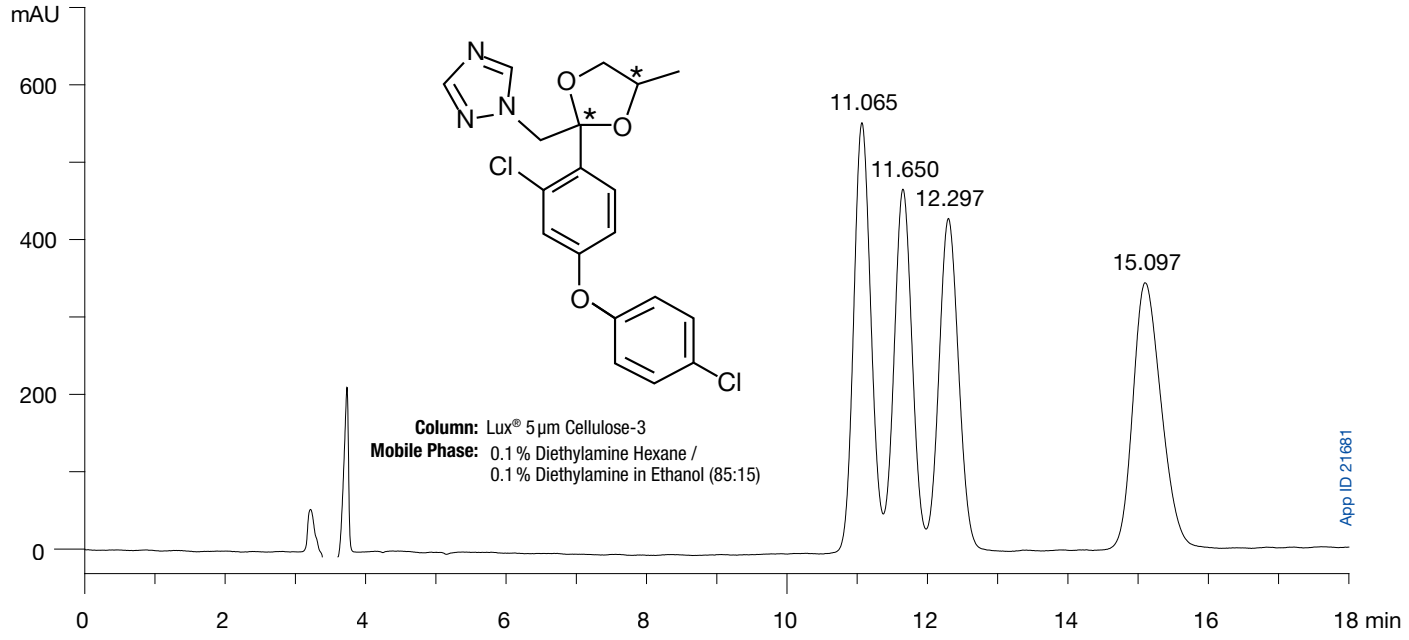


Figure 6.
Stereoselective HPLC analysis on the stereoisomers mix of Propiconazole

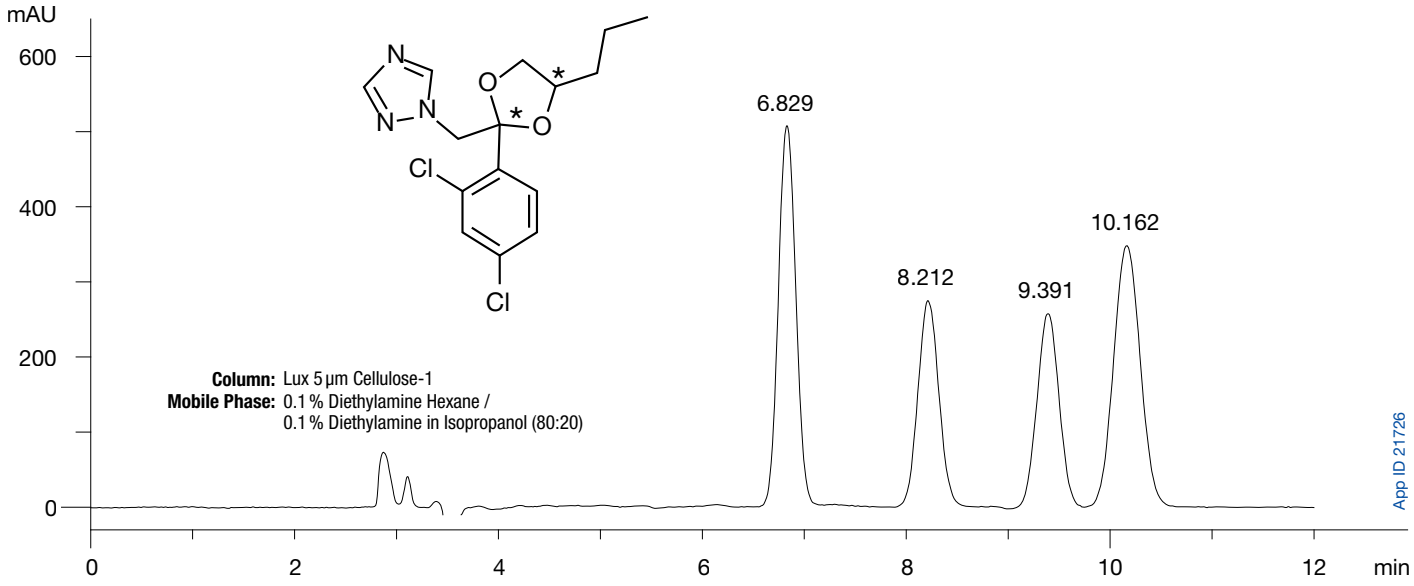
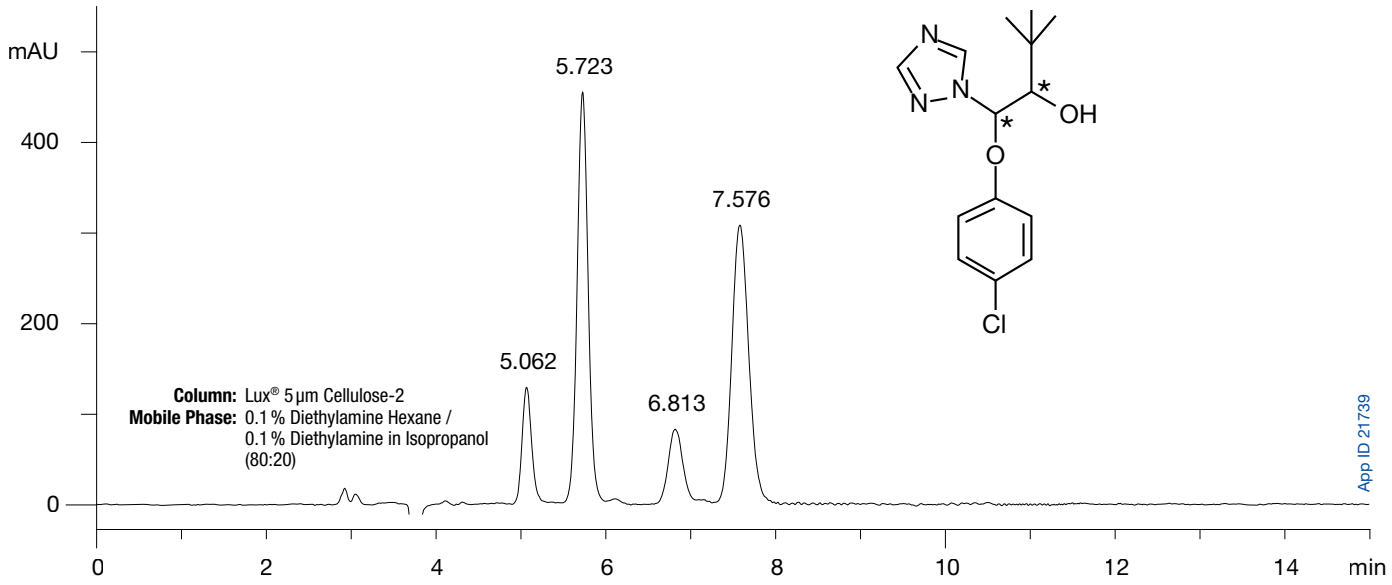


Figure 7.
Stereoselective HPLC analysis on the stereoisomers mix of Triadimenol



Conclusion

In this application note, we described the enantiomeric and diastereoisomeric resolution of five fungicide agents containing 2 stereogenic centers using Lux polysaccharide-based chiral stationary phases. All stereoisomeric separations reported showed baseline resolution between all stereoisomers with run time below 25 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. These analytical and preparative products can also be used under SFC mode for higher throughput.[8]

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