Achiral SFC: No C18 Equivalent, No Problem

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Introduction

For several years, a large amount of research has been conducted in the search for a universal achiral SFC column. This universal column would be the SFC equivalent to the C18 column for reversed phase chromatography. These efforts have evaluated long lists of probes, many forms of column chemistry, and employed sophisticated statistical treatment of screening data. However, to date there is still no magic universal column for SFC.

A universal column for achiral SFC applications would be convenient but does SFC need to have a universal column? Chiral chromatography in the pharmaceutical industry can be credited with shaping the entire SFC industry into its current position. Chiral chromatography does not have a universal column. SFC has excelled in this separation science niche because it is particularly effective at screening multiple columns and different eluent compositions. The same approach can be applied to achiral chromatography. SFC can be effectively applied to achiral applications when a limited set of columns are known to be applicable for the diverse range of compounds suitable for SFC.

The work presented here will discuss the development of achiral applications for SFC. The focus will be on compounds relevant to the pharmaceutical industry. Columns typically utilized for normal phase, reversed phase, and chiral applications will be used for achiral SFC applications.

Discussion

HPLC is the most common chromatographic methodology for achiral applications. One of the strongest advantages that HPLC has over other chromatographic techniques is the diverse applicability of the C18 stationary phase. Method development is streamlined when the first column selected works most of the time. Many achiral chromatographic applications could be accomplished with either SFC or HPLC but there is not a stationary phase for SFC that is as widely applicable as the C18 column is for HPLC. The advantage SFC does have over HPLC is speed. Column screening can take a significant amount of time for HPLC because of limitations due to eluent viscosity. However, column screening with SFC can be accomplished much faster with the low viscosity eluents found in the technique.

SFC has excelled in this separation science niche because it is particularly effective at screening multiple columns and different eluent compositions. The same approach can be applied to achiral chromatography. SFC can be effectively applied to achiral applications when a limited set of columns are known to be applicable for the diverse range of compounds suitable for SFC.

SFC Column Chemistries Used in This Study

Achiral Phases

Luna® NH2

Luna HILIC (cross-linked diol)

Luna Silica(2)

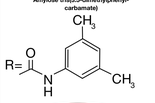
Synergi™ Polar-RP (ether linked phenyl with polar endcapping)

Kinetex® Biphenyl

Kinetex F5 (pentafluorophenyl propyl)

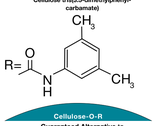
Chiral Phases

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



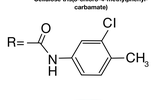
Amylose-O-R
Guaranteed Alternative to CHIRALPAK® AD®, AD-H®, AD-3, AD-RH®, and AD-3R

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



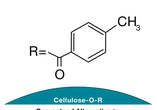
Cellulose-O-R
Guaranteed Alternative to CHIRALCEL® OD®, OD-H®, OD-3, OD-RH®, and OD-3R

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



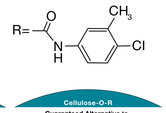
Cellulose-O-R
Guaranteed Alternative to CHIRALCEL OZ, OZ-H®, OZ-3, OZ-RH, and OZ-3R

Lux Cellulose-3
Cellulose tris(4-methylbenzoate)



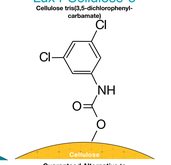
Cellulose-O-R
Guaranteed Alternative to CHIRALCEL OJ®, OJ-H®, OJ-3, OJ-RH®, and OJ-3R

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



Cellulose-O-R
Guaranteed Alternative to CHIRALCEL OX, OX-H, OX-3, OX-RH, and OX-3R

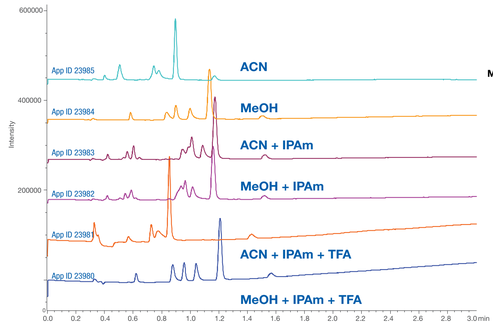
Lux i-Cellulose-5
Cellulose tris(3,5-dichlorophenylcarbamate)



Cellulose
Guaranteed Alternative to CHIRALPAK IC® and IC-3

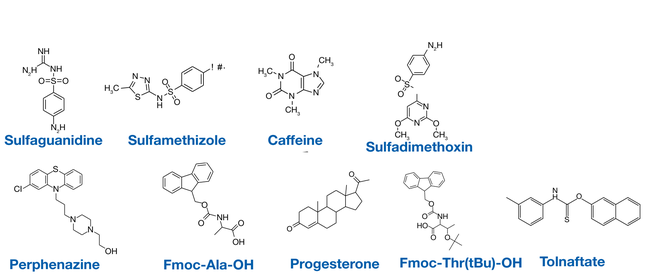
Example 1: Pharmaceutically Related Compounds

Step 1: Screen Co-Solvents on Kinetex Biphenyl

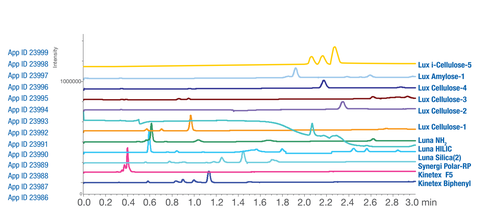


Column: Kinetex 2.6 µm Biphenyl
Dimensions: 150 x 3.0 mm
Part No.: 00F-4622-Y0
Mobile Phase: A: Carbon Dioxide
B: As described
Gradient:

Time (min)	% B
0	5
2.5	25
3	25



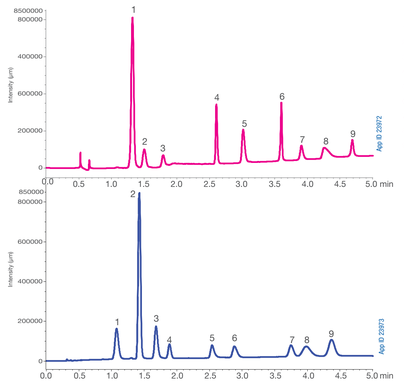
Step 2: Column Screen



Column: As described
Dimensions: 150 x 3.0 mm
Mobile Phase: A: Carbon Dioxide
B: Methanol
Gradient:

Time (min)	% B
0	5
2.5	25
3	25

Step 3: Method Optimization



Column: Luna 3 µm HILIC
Dimensions: 150 x 3.0 mm
Part No.: 00F-4449-Y0
Mobile Phase: A: Carbon Dioxide
B: Methanol
Gradient:

Time (min)	% B
0	1
1	1
5	40

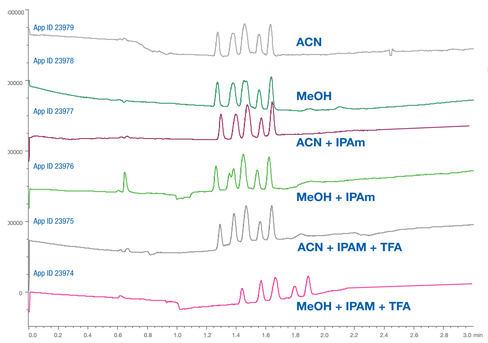
Column: Lux 3 µm Amylose-1
Dimensions: 150 x 3.0 mm
Part No.: 00F-4729-Y0
Mobile Phase: A: Carbon Dioxide
B: Methanol
Gradient:

Time (min)	% B
0	10
5	25

Example 1: Discussion
From **Step 1** it was determined that methanol was a much better co-solvent than acetonitrile and the use of additives were not needed.
From **Step 2** it was determined that Luna HILIC and Lux Amylose-1 were promising column choices.
After optimization in **Step 3**, methodology was obtained with both columns that have different selectivities for these compounds.

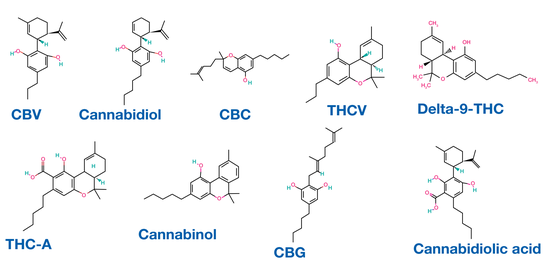
Example 2: Cannabinoid Compounds

Step 1: Screen Co-Solvents on Kinetex Biphenyl

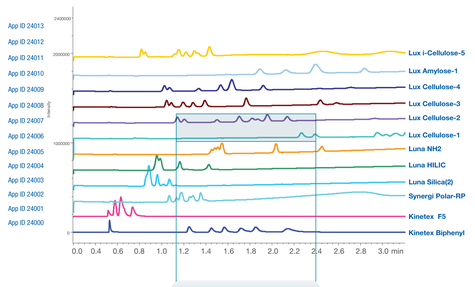


Column: Kinetex 2.6 µm Biphenyl
Dimensions: 150 x 3.0 mm
Part No.: 00F-4622-Y0
Mobile Phase: A: Carbon Dioxide
B: As described
Gradient:

Time (min)	% B
0	5
2.5	25
3	25



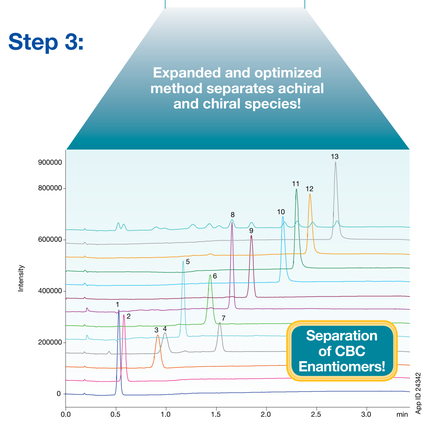
Step 2: Column Screen



Column: As described
Dimensions: 150 x 3.0 mm
Mobile Phase: A: Carbon Dioxide
B: Methanol
Gradient:

Time (min)	% B
0	5
2.5	25
3	25

Step 3:



Column: Lux 3 µm Cellulose-2
Dimensions: 150 x 3.0 mm
Part No.: 00F-4456-Y0
Mobile Phase: A: Carbon Dioxide
B: Methanol
Gradient:

Time (min)	% B
0	4
3	25
3.5	25

Flow Rate: 5 mL/min
Detection: UV @ 220 nm
Temperature: 40 °C
Sample: Cannabinoid mix of 12

1. CBDV	8. THCV
2. CBN	9. CBC
3. Delta-8-THC	10. CBDA
4. CBC (Enantiomer 1)	11. CBDVA
5. CBD	12. THCA
6. Delta-9-THC	13. CBGA
7. CBC (Enantiomer 2)	

Example 2: Discussion
From **Step 1** it was determined that methanol was not much different than acetonitrile as a co-solvent and that the use of additives were not needed.
From **Step 2** it was determined that Kinetex Biphenyl and Lux Cellulose-2 were promising column choices.
After optimization in **Step 3**, methodology was obtained with both columns that have different selectivities for these compounds.

Experimental

Reagents
- Carbon dioxide, food grade from Praxair®
- Methanol (MeOH), acetonitrile (ACN), trifluoroacetic acid (TFA), and isopropylamine (IPAm), HPLC grade from Sigma-Aldrich®
- Example 1: Standards from Sigma-Aldrich
- Example 2: Cannabinoid standards from Cerilliant®

Instruments
"SF-4000" Analytical SFC from JASCO®

Conditions for all examples
Flow Rate: 3 mL/min
Column Temperature: 40 °C
Detection: UV @ 220 nm
Backpressure: 120 bar

Co-Solvents

The most commonly used co-solvent for SFC is methanol. Acetonitrile can be used for SFC methodology but is typically a weaker solvent and is less commonly used. However, there can be significant selectivity differences between these solvents that allow resolution of critical pairs. SFC is similar to HPLC with respect to peak shape issues. Some compounds will have fronting or tailing peak shapes and these can often be improved by the addition of an additive to the co-solvent.

The work presented here evaluated 6 different co-solvents. Methanol and acetonitrile were each used as 3 different forms. They were either used without any additives, with 0.1 % Isopropylamine or a mixture of 0.1 % Isopropylamine and 0.1 % Trifluoroacetic acid.

Basic 3-Step Screen

Step 1. Screen Co-Solvents

Use an appropriate sample that has a representative chromatographic profile

Use a single column; this work used a Kinetex core-shell Biphenyl LC column

Evaluate additives, this work used methanol to evaluate acidic, basic, acid/base mixed, and without any additives

Use a fast gradient, an example would be 5 % to 25 % over 2 min with a 30 second hold

Interpret results by comparing peak shape, retention and how many peaks were observed

Evaluate other solvents such as acetonitrile, isopropanol, or mixtures if necessary

Select the most promising conditions and move on to **Step 2**

Step 2. Column Screening

Use the best co-solvent additive combination found in Step 1

Evaluate columns that have been previously successful with achiral SFC

Use a gradient similar to the one used in **Step 1**

Interpret results by comparing peak shape, retention and how many peaks were observed

If nothing is promising, select other column chemistries and repeat

If promising conditions are found, move on to **Step 3**

Step 3. Method Optimization

Expand the gradient around the observed peaks
- If all of the peaks are early, lower the final gradient % co-solvent
- If all of the peaks are late, raise the initial gradient % co-solvent
- If the peaks are very close, extend the gradient over a longer period of time

Determine if a gradient is needed

Evaluate if the chromatographic selectivity is dependent on the eluent density by screening with backpressure set higher and lower than typical; 20 – 30 bar difference is suitable

Finalize the gradient slope (if necessary)
- If the peaks are well resolved, shorten the time for the gradient
- If the peaks need more resolution, lengthen the time for the gradient

Conclusion

SFC is very effective for achiral applications even without a "universal" SFC achiral column. The ability to quickly screen multiple column and eluent combinations allows for methodology to be quickly and easily developed. Polysaccharide chiral columns can be very effective for Achiral SFC separations and should be part of a comprehensive SFC achiral development plan.

Acknowledgements

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