SELECTING THE RIGHT SFC COLUMN

FOR HIGH SPEED AND HIGH RESOLUTION

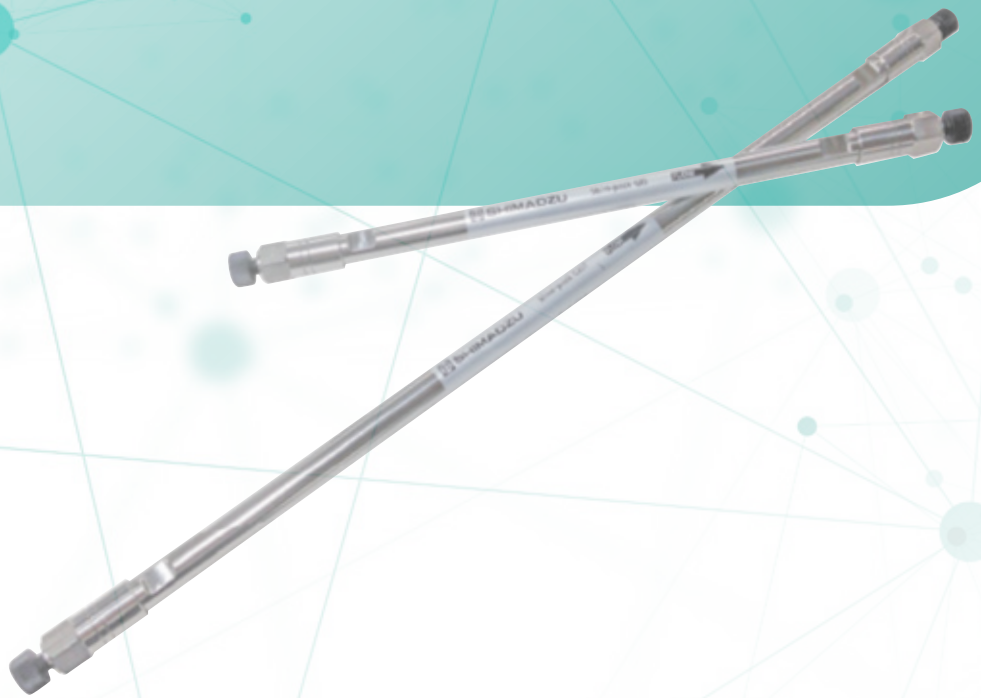
SHIMADZU

Excellence in Science

Supercritical fluid chromatography (SFC)

is a valuable technique for analytical and preparative separation of compounds for drug synthesis because of its fast separations and analysis time, high sensitivity and reduced solvent consumption. In addition, robust SFC systems give you the opportunity to integrate analytical UHPLC/SFC and easily access mass spectrometry, all supported by a unified software platform.

This eBook examines column options for accurately and efficiently separating a variety of pharmaceutical compounds using SFC.



SFC Column Fundamentals

SFC columns are generally compatible with fittings used in HPLC systems, but it is important to confirm the pressure capacity and other requirements for actual use with respective column manufacturers. Columns used in SFC must be able to withstand high pressures at both the inlet and outlet end because of the back pressure regulators that maintain SFC conditions in the chromatographic system and ensure the CO_2 is kept in a supercritical fluid state.

Polymer-based columns require particular care because the pressure increases during use as the stationary phase expands. In addition, minimizing the elastomeric materials in the column will assure leak-free column performance.

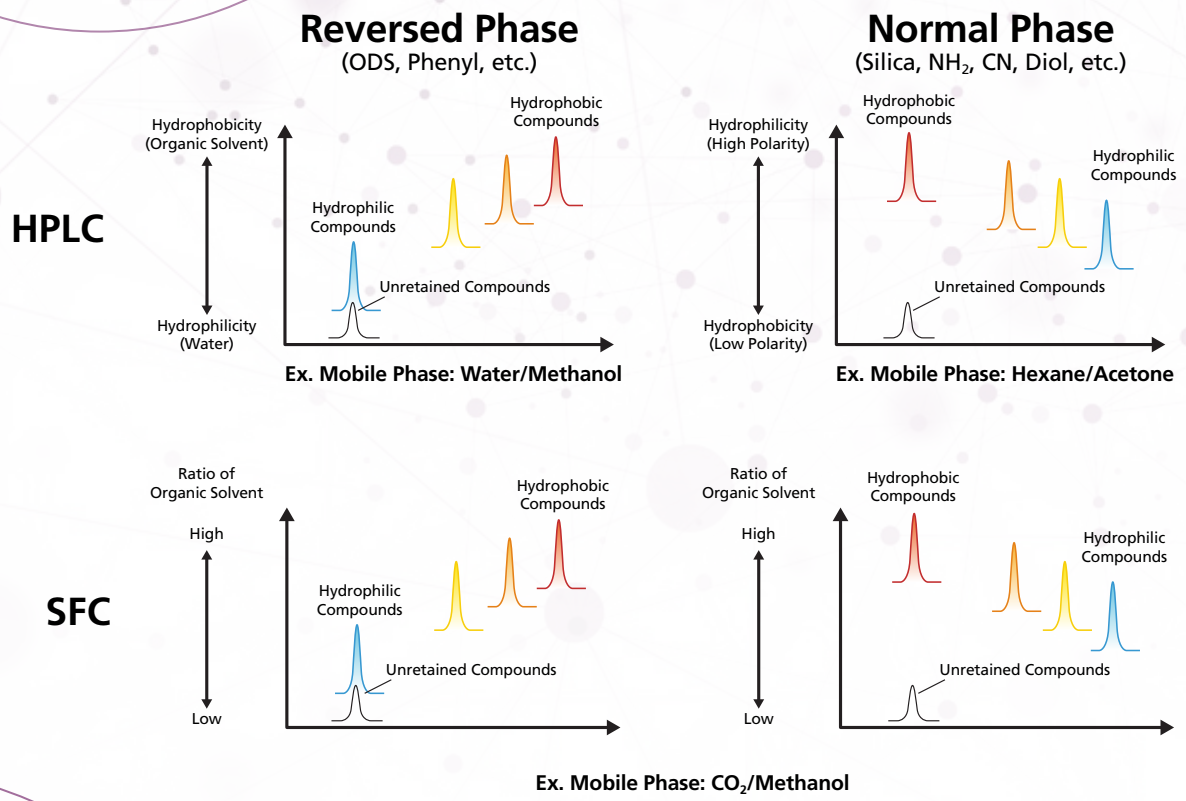
Because supercritical CO_2 has about the same polarity as hexane, the main SFC separation modes will behave similarly to normal-phase chromatography. Columns with properties of both normal-phase (such as silica, diol, and CN) and reversed-phase (such as C18, cholesteryl, and phenyl) can be used, unlike HPLC.

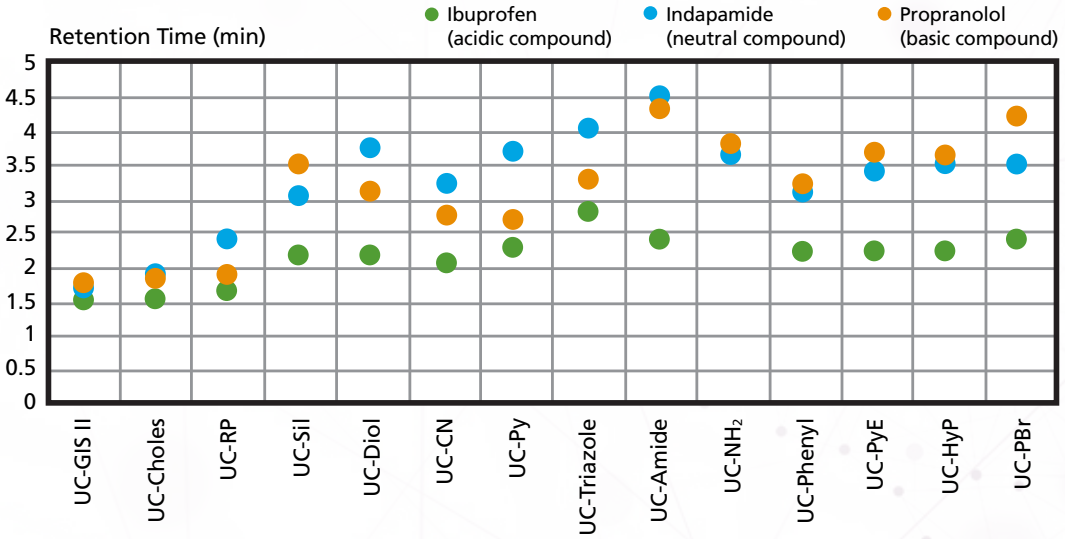
The supercritical CO_2 used for SFC has high mobile phase diffusivity and low molecular density, making it more prone to secondary interactions than HPLC, which can lead to significant changes in separation behavior depending on the column stationary phase. Consequently, to determine optimal conditions, it is important to consider analytical conditions using a wide variety of stationary phase types.

Although it has low polarity, supercritical CO_2 can be mixed with highly polar solvents. Therefore, when optimizing the mobile phase and stationary phase for SFC, normal-phase columns can be replaced with a reversed-phase column without changing the type or composition of mobile phase or modifier.

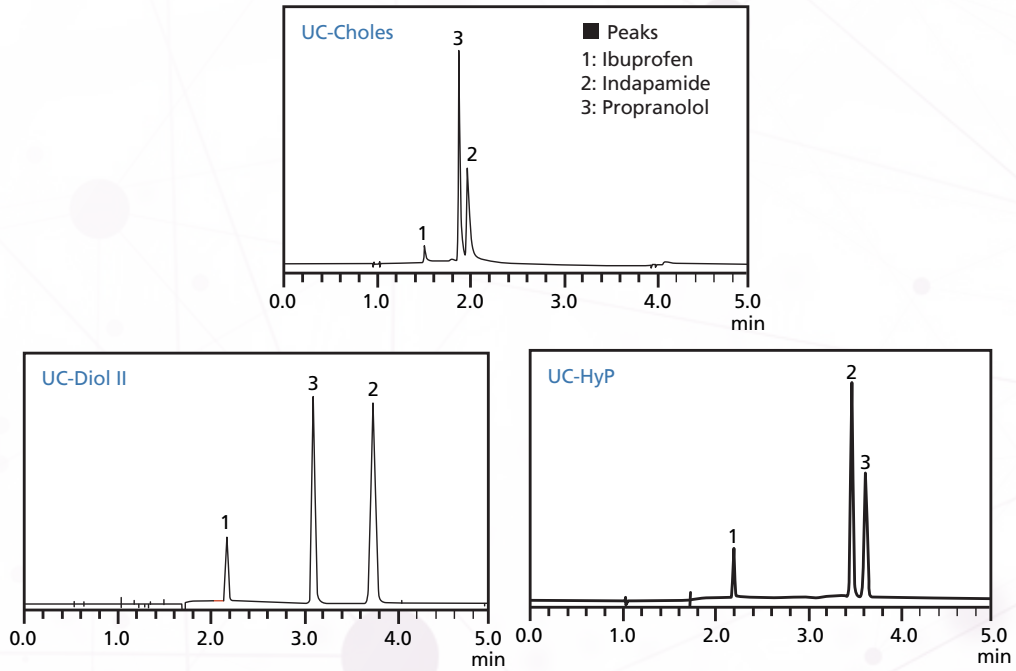
The figure to the right shows a comparison of the elution order for HPLC and SFC. For HPLC, mobile phases are matched to either the reversed-phase or normal-phase separation mode based on mobile phase properties. This involves purging the solvent from inside the system each time the separation mode is changed.

For SFC, the same mobile phase conditions can be used for either the reversed-phase or normal-phase separation modes, which means separation results can be checked by simply replacing the column.





Retention Behavior of Respective Shim-pack UC Series Products.



Differences in Retention Behavior for Respective Stationary Phases (Normal Phase for Diol, Normal Phase + Static Electric Interaction for HyP and Hydrophobic Interaction for Choles)

For SFC no columns are available that offer broad applicability, such as the C18 column does for HPLC reversed-phase chromatography. Because supercritical CO_2 can penetrate tiny pores more easily, it promotes higher interaction with the stationary phase. Hence, SFC offers the possibility of separating isomers or other compounds that are difficult to separate by HPLC. In particular, columns with unique or multiple interactive effects can help improve separation using SFC.

The following figures show an index of elution order and retention behavior for a variety of columns used to analyze ibuprofen (acidic compound), indapamide (neutral compound) and propranolol (basic compound), as well as chromatograms obtained using diol group, hydroxyphenyl group and cholesteryl group columns.

SFC Column Considerations

1. Due to the pressure on both the inlet and outlet ends, selecting the best hardware for SFC preparative columns is particularly challenging. A frit or bed failure in a preparative SFC column can send packing material into the preparative instrument. Also, rapid CO_2 decompression can easily damage preparative columns. Look for hardware and techniques that ensure packed-bed stability for the columns.

2. Using columns for both HPLC and SFC requires special care regarding purging column liquids after the completion of analyses. After using the column for SFC analysis, reduce the pressure inside the column to vaporize any CO_2 in the column or purge it with methanol or ethanol before disconnecting the column.

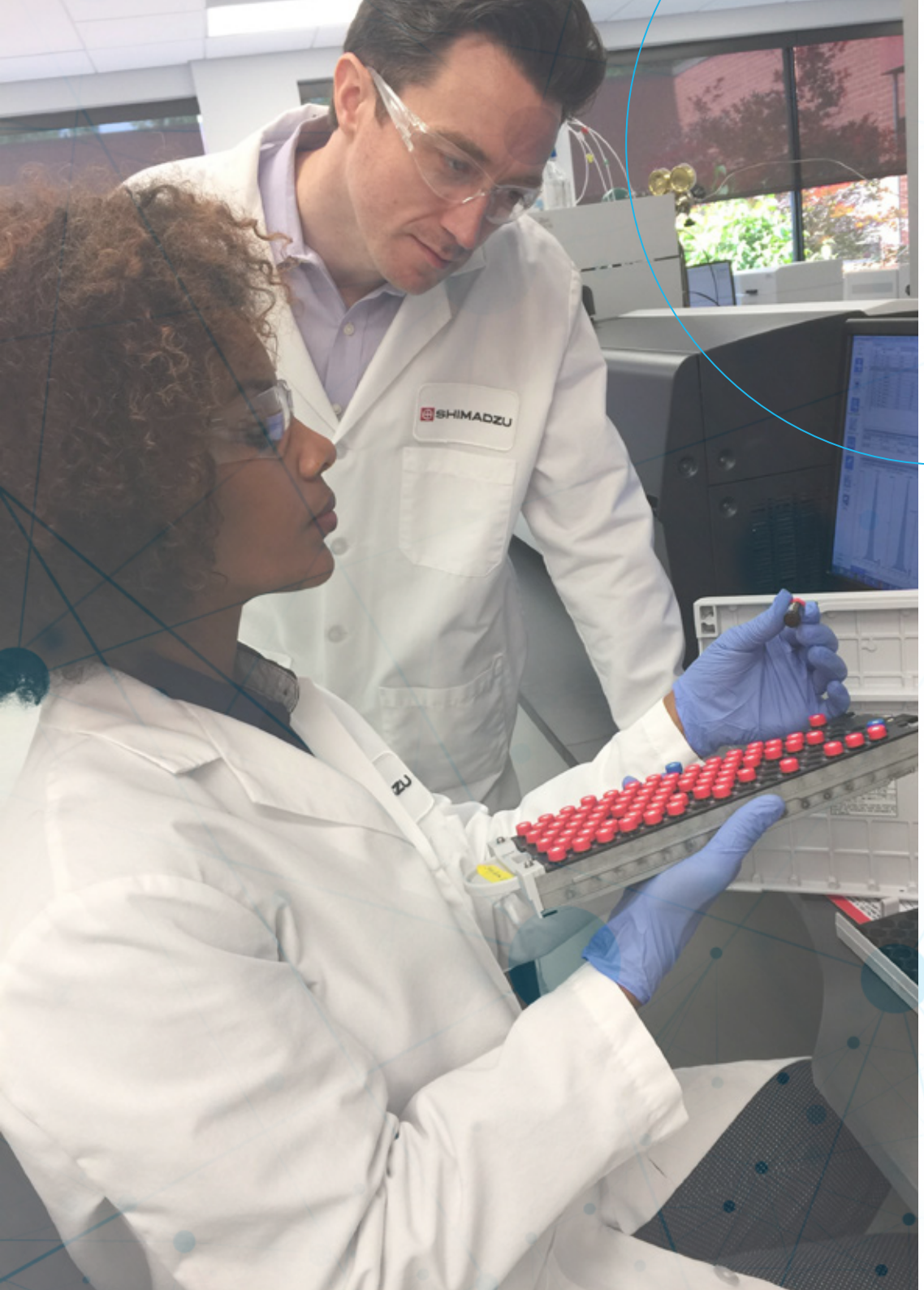
3. Disconnecting the column with only supercritical CO_2 dries out the packing material due to evaporation of liquid in the column. If that column is then used for HPLC, the high viscosity of the solvent used for the LC mobile phase, such as methanol or water, will cause surface tension around pores in the stationary phase that prevents penetration into the pores and interaction with functional groups.

4. Because supercritical fluid used for SFC analysis has lower viscosity than mobile phase solvents used for HPLC, it can penetrate inside pores and interact with functional groups without being affected by surface tension, even if not filled with solvent.

5. If a column used for HPLC analysis is used for SFC, any water in the column must be thoroughly purged with methanol or ethanol, because the water will not mix with the supercritical CO_2.

6. For SFC, the same mobile phase parameters can be used for everything from normal-phase columns to reversed-phase columns. First fix the mobile phase parameters, then check separation using multiple columns.

7. The multiple columns, solvent delivery parameters and separation parameters (such as temperature and pressure) result in a large number of possible parameter combinations. To reduce the amount of work involved in considering separation parameters, use a method scouting system to automatically set and execute respective parameter combinations.



Nexera UC SFC Column Chemistries

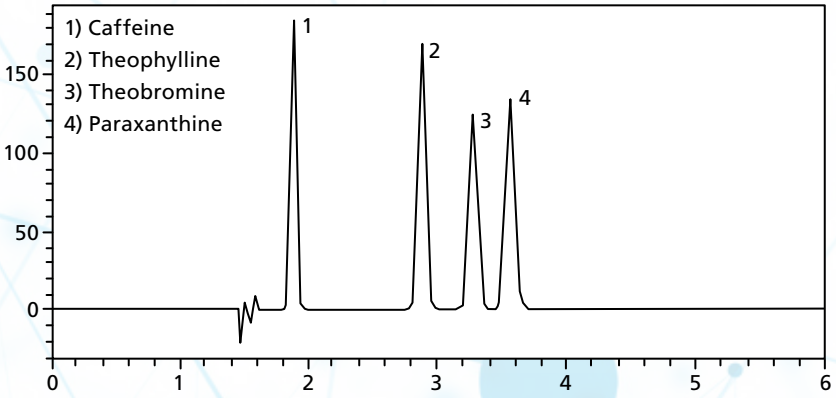
Nexera UC SFC columns are designed specifically for use in SFC. The Nexera UC base silica is metal-free ultra-high-purity chromatographic media that is pressure stable and specifically engineered for high-performance SFC separations. The surface is treated to produce maximum SFC separation interactions and loading capacity while maintaining superior peak shape performance for many analytes.

Nexera UC SFC column chemistries demonstrate unique selectivity. All of these materials are available in sub-2 µm, 5 µm, 7 µm, 10 µm and 15 µm particle size. Columns from 1 mm ID (various analytical sizes) to 100 mm ID (various preparative sizes) are available.

The following is a list of Nexera UC SFC columns based on separation objectives.

For retention and rapid separation of analytes containing strong amine groups:

The Nexera UC Basic column is based on imidazole chemistry and provides a highly basic character for this stationary phase. SFC separation of amines normally requires the addition of an amine to the mobile phase, but a Nexera UC Basic column does not need the addition of these peak shape modifiers. Mobile phase composition and fraction collection is greatly simplified without the use of amino additives. The chromatogram shown below demonstrates the superior peak shape performance and separation capacity obtainable with the Nexera UC Basic column with SFC. Nexera UC Basic is available for analytical and preparative column formats in particle sizes from 1.8 µm to 20 µm.

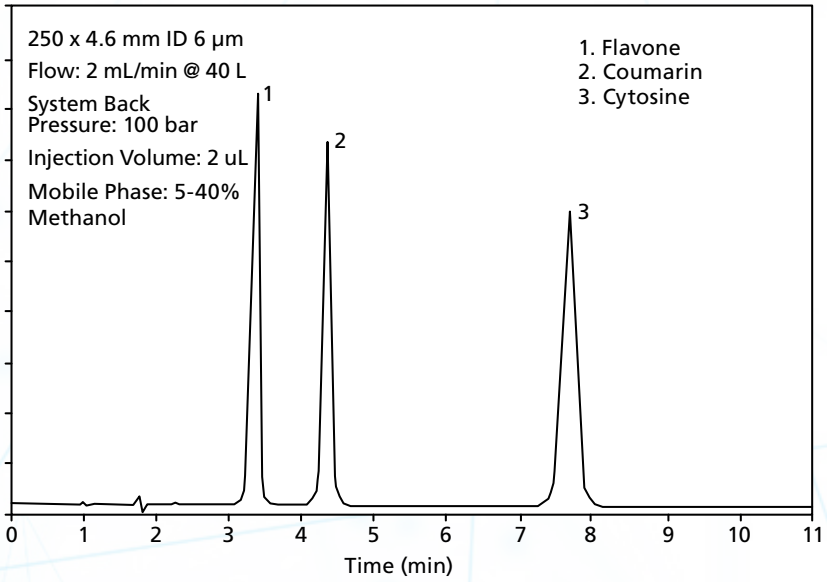


Column: Nexera UC Basic 5 µm, 4.6 x 250 mm
Flow: CO_2 2.7 mL/min, Methanol 0.3 mL/min
BPR: 400 bar
Detection: UV @ 280 nm

For the separation of geometrical isomers and diastereomers:

Nexera UC PFP is the column of choice in separating analytes that contain aromatic groups, polarizable electrons and conjugate systems. It is also useful for the separation of halogenated compounds.

Nexera UC Nitro is a highly unique phase and used for separating analytes that contain aromatic groups, polarizable electrons and conjugate systems. Please see an example chromatogram below.

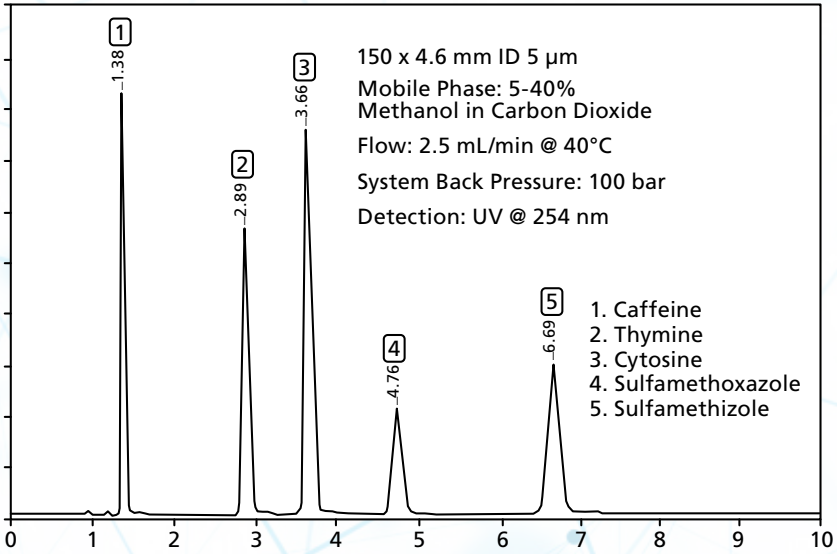


In many cases Nexera UC PFP provides orthogonal separations when compared to Nexera UC Nitro.

For the retention and rapid separation of an amine group containing analytes:

Nexera UC Ethyl Pyridine column is ideal for compounds that are functionalized with strong amine groups. Excellent peak shape during separation is achieved without the addition of an amine to the mobile phase as a peak shape modifier.

Nexera UC DEAP provides retention and rapid separation of chemical compounds containing strong amine groups without the need for an amine modifier, simplifying mobile phase composition and fraction collection. It has greater retention for amines than Nexera UC Ethyl Pyridine. Please see an example chromatogram below.

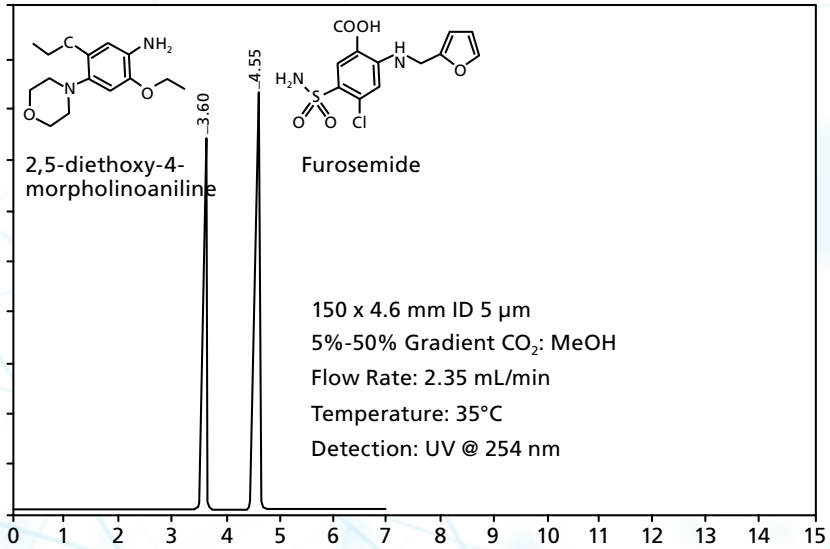


For diastereomers separations and non-polar compound separation:

Nexera UC Naphthyl is a naphthalene-based SFC material with very high bonding density and intrinsic base deactivation due to a rigid structure that enables the shape selectivity needed for many diastereomeric separations. Additionally, this phase exhibits strong π-π interaction and charge transfer interactions and performs well for diastereomers separations and non-polar compound separation. The unique properties of Nexera UC Naphthyl place its selectivity between graphitized carbon and alkyl type stationary phases.

For the separation of target analytes functionalized with both amine bases and acidic groups:

Nexera UC Pyridyl Amide provides flexibility and simplifies mobile phase composition and fraction collection without the use of amino or TFA additives. Please see an example chromatogram below.

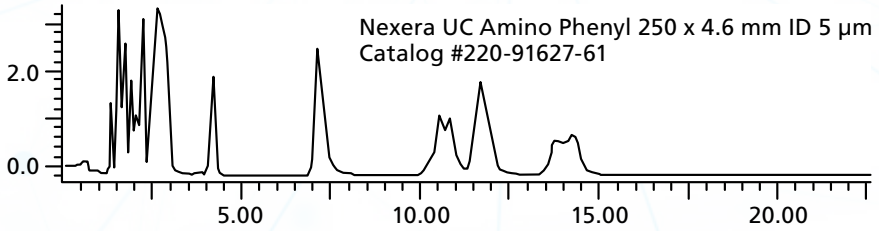


Nexera UC Ethyl Pyridine II SFC column is ideally suited for the retention and rapid separation of analytes containing amine as well as acid groups.

Nexera UC 4-Ethyl Pyridine is an alternative phase that provides different selectivity to Nexera UC Ethyl Pyridine (2-ethyl pyridine).

For the separation of amines, alcohols and acids without the use of additives:

Nexera UC Amino Phenyl works well in normal phase mixed mode offering π-π interaction and exhibits good base deactivation.



15% Methanol in CO_2
Pressure: 100 bar
Flow: 3 mL/min
Detection: PDA

1. Butyl benzoate
2. 4-nitrotoluene
3. 2-nitrophenol
4. Caffeine
5. Antipyren
6. Oxprenolol
7. Niacinamide
8. Sorbic acid
9. Naphthalene
10. Ibuprofen
11. N-benzylbenzamide
12. 1-aminoisoquinoline
13. Propranolol

For high-performance SFC separations:

Nexera UC Silica is a metal-free ultra-high purity chromatographic media that is pressure stable. The surface is treated to produce maximum separation interactions and loading capacity while maintaining superior peak shape performance for many analytes. Nexera UC Silica is available for analytical and preparative column formats in particle sizes from 1.8 µm to 20 µm.

Sample Considerations

Care should be taken with large injection volumes, particularly for preparative SFC. In these situations, the injected sample can plug the column and shut down the chromatographic system. There are several ways of avoiding this "crashing out," such as using a mobile phase co-solvent like acetonitrile or methanol or mixing the injected sample with solvent that is more compatible with CO_2.

Shim-pack™ UC Series Columns

Shim-pack™ UC series columns are designed specifically for Nexera UC series SFC systems. When using supercritical fluids for analysis, separation behavior can vary significantly depending on the type of solid phase. To optimize separation, a variety of columns should be used to determine the parameter settings. The extensive choice of column sizes available means that operations can be scaled up seamlessly from analytical SFC to preparative SFC.

The Shim-pack UC series columns offers an assortment of stationary phases for separating a variety of compounds using SFC. The series includes 20 types of packing materials and a range of particle and column sizes that can be selected based on the analyte.

	Functional Group
Shim-pack UC-ODS	Octadecyl group
Shim-pack UC-GIS II*	Octadecyl group
Shim-pack UC-RP*	Octadecyl + polar functional group
Shim-pack UC-Sil*	—
Shim-pack UC-Sil II	—
Shim-pack UC-Diol*	Diol group
Shim-pack UC-Diol II	Diol group
Shim-pack UC-CN*	Cyanopropyl group
Shim-pack UC-Py	Pyridinyl group
Shim-pack UC-PolyVP	Poly (4-vinylpyridine) group

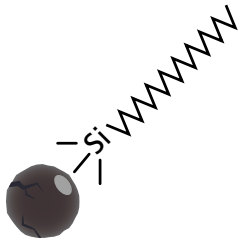
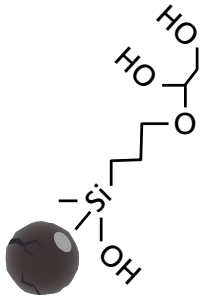
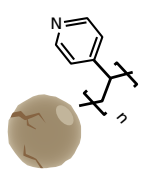
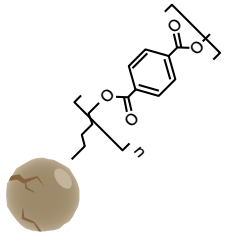
	Functional Group
Shim-pack UC-Triazole	Triazole group
Shim-pack UC-Amide*	Carbamoyl group
Shim-pack UC-NH_2*	Aminopropyl group
Shim-pack UC-Choles	Cholesteryl group
Shim-pack UC-PolyBT	Polybutylene terephthalate (coated on silica gel)
Shim-pack UC-Phenyl*	Phenyl group
Shim-pack UC-NaE	Naphtylethyl group
Shim-pack UC-PyE	Pyrenylethyl group
Shim-pack UC-HyP	3-hydroxyphenyl group
Shim-pack UC-PBr	Pentabromobenzyl group

*Only available in 2.1 mm and 4.6 mm ID column sizes.

Selecting SFC Columns

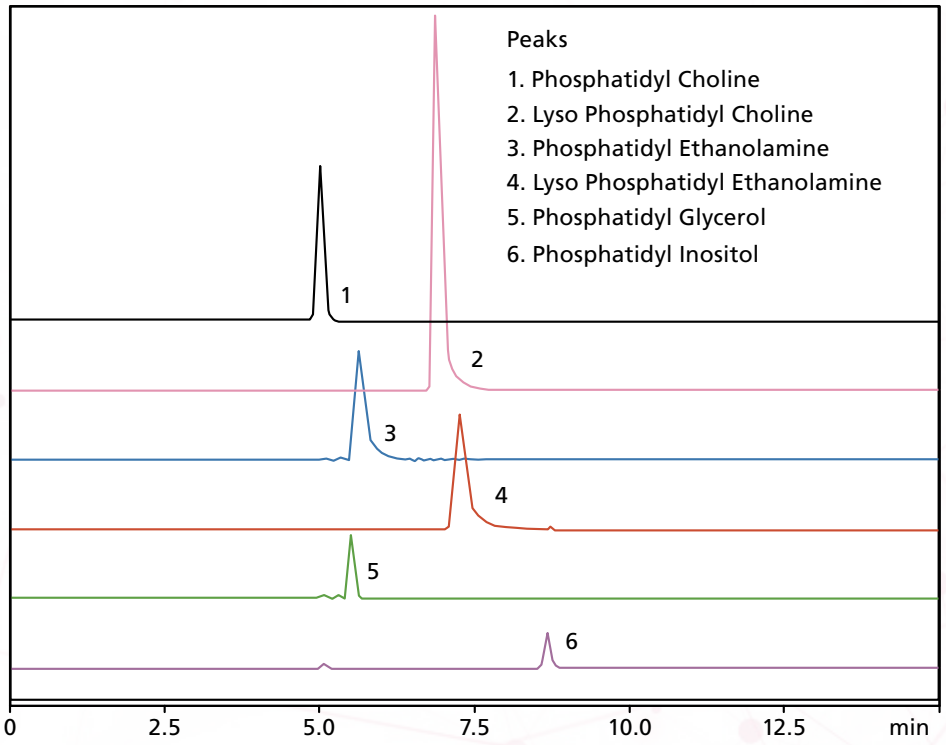
Because normal phase separation is the main separation mode used for SFC, normal phase UC-Diol and UC-Diol II are the most commonly used columns. They are followed by UC-Py columns, which exhibit similar behavior to ethylpyridine-based columns.

HPLC involves using mobile phases with very different compositions for reverse phase and normal phase analysis, such as water-based versus non-water-based mobile phases. In contrast, SFC uses a mixture of supercritical CO_2 and a modifier (an organic solvent such as methanol) regardless of the stationary phase used. Therefore, the same mobile phase composition can be used for serial analysis through all columns. Column scouting is effective by using the following set of 6 columns, each providing a different separation selectivity.

	6 Columns Set					
	UC-ODS	UC-Sil II	UC-Diol II	UC-PolyVP	UC-PolyBT	UC-PBr
Chemistry						
Features	The separation mode is reversed phase. Retention is provided through hydrophobic interaction.	This is excellent for retention of basic compounds and recognition of their tertiary structures.	The separation mode is normal phase. This inhibits nonspecific interactions.	A favorable peak shape is obtained even without acid-base additives.	This is excellent for resolving aromatic compounds through π-π interactions.	With ODS, separation of poorly retained compounds is improved.

UC-Diol and UC-Diol II columns offer excellent general applicability for analyzing a wide variety of compounds, from phospholipids and other lipids to highly polar peptide compounds. However, a column with an ODS group stationary phase, such as the Shim-pack UC-ODS and Shim-pack UC-GIS II, must be used to separate phospholipids by molecular species with similar modifier parameters.

You can separate isomers and other compounds by SFC that are difficult to separate by HPLC. Columns with specific or multiple interaction modes may help improve separation. UC-PolyBT, with its high planar recognition capacity, and UC-PBr, with its dispersion power with Br, are useful.



Analysis of Phospholipids (UC-Diol/UC-Diol II)

Columns for SFC Achiral Analysis

SFC sample preparation and analysis has grown to include achiral separations to support drug discovery and development. The Nexera UC series SFC columns include 14 achiral types of stationary phases with a range of sizes to meet diverse research and development needs. Columns are available in sub-2 µm, 3 µm and 5 µm particle sizes in both analytical and preparative scale.

- Nexera UC SFC achiral separation columns include:
- Amine columns with a high-density NH_2 bonded material for SFC analysis requiring higher sample loading
- Amino Phenyl columns for the separation of amines, alcohols and acids without the use of additives
- Basic columns for high-speed separation of chemicals containing amine groups
- Cyano columns with high-surface area for higher sample loading
- DEAP (diethylaminopropyl) columns for separation of compounds that would normally require the addition of an amine modifying agent to the mobile phase
- Diol columns with high-density diol surface coverage for better and more reproducible separations compared to conventional unbonded silica
- Ethyl Pyridine columns for chemicals that are functionalized with strong amine groups to eliminate the need for amino additives

- Ethyl Pyridine II columns for the retention and rapid separation of chemicals containing acidic groups
- 4-Ethyl Pyridine columns for providing different selectivity to the Ethyl Pyridine (2-ethyl pyridine) columns
- HILIC columns composed of a polyhydroxylated polymer that is coated and bound for higher sample loading
- Naphthyl columns for diastereomer separations as well as non-polar compounds
- Nitro columns for the separation of geometrical isomers as well as diastereomers
- PFP (pentafluorophenyl) columns for the separation of geometrical isomers as well as diastereomers
- Pyridyl Amide columns for separation of compounds that would normally require the addition of TFA or an amine modifying agent to the mobile phase
- Silica column substrates are metal-free and ultra-high purity for high-performance SFC applications

Columns for SFC Chiral Analysis

The supercritical CO_2 used in SFC is nonpolar and has similar properties to n-hexane normally used as a mobile phase in normal-phase chromatography. Therefore, it is possible to migrate chiral separation methods from conventional normal-phase chromatography to SFC. Doing so, however, requires selecting from a wide variety of columns and modifiers during optimization of chiral compound separation parameters for SFC.

Incorporate as many chiral stationary phases (CSPs) into the screening as possible. Use both coded and immobilized polysaccharides, brush type, proteins and others. Particle size is another consideration. Smaller particles tend to give better resolution, but with increased pressure on the system. To achieve efficient resolution, make sure the system can run the pressures required for those particle sizes. Increasing the length of the column can give additional retentive properties but with additional runtime.

Normal mobile phase, reverse phase, polar organic, SFC and extended range can all be used, depending on the solubility of the sample. However, note that they can give very different results even when used on an identical CSP.

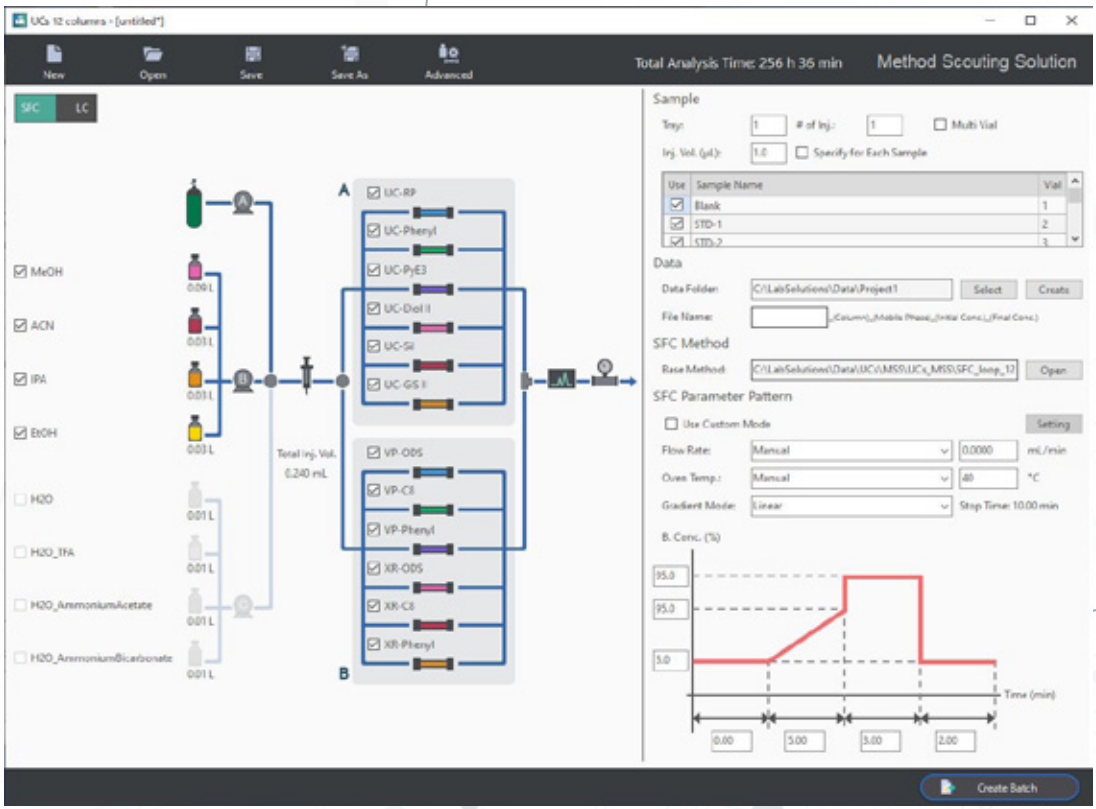
Modifiers can be used to adjust peak shape and typically are sample dependent. Also, determine whether to run gradient or isocratic elution. Gradient runs tend to reduce the number of injections. However, care must be taken not to miss separations, especially when running ballistic gradients. Be sure to re-equilibrate the column in between sample runs. Isocratic runs tend to require more injections to run, but they are less likely to run tight separations, and running equilibration between the samples is not necessary.

If analysis needs to be optimized after the primary screening, consider changing the mobile phase composition, augmenting the modifier from one organic base to another, changing the temperature or increasing the column length. Use a centralized composite way of augmenting one of these parameters, while holding the others constant.

If the process does not provide an optimized result, return to the secondary prime, secondary CSP/mobile phase hit and repeat the process until an optimized result is attained. The more compounds or mobile phase combinations tried, the higher probability of success.

The following is an example of using a Nexera UC chiral screening system to determine the optimal chiral separation parameters for omeprazole. The Nexera UC chiral screening system can automatically switch between up to twelve columns, four types of modifiers and various mixture ratios to automatically optimize from a large number of possible separation parameters. Each parameter setting is specified using Shimadzu's Method Scouting Solution software shown in the figure to the right.

Based on the chromatogram obtained, optional software is used to evaluate separation and rank the optimized parameters (see figure below). The software automatically identifies all chromatograms with resolution greater than a specified criterion (1.5 in this case) and then ranks the resolution in those chromatograms



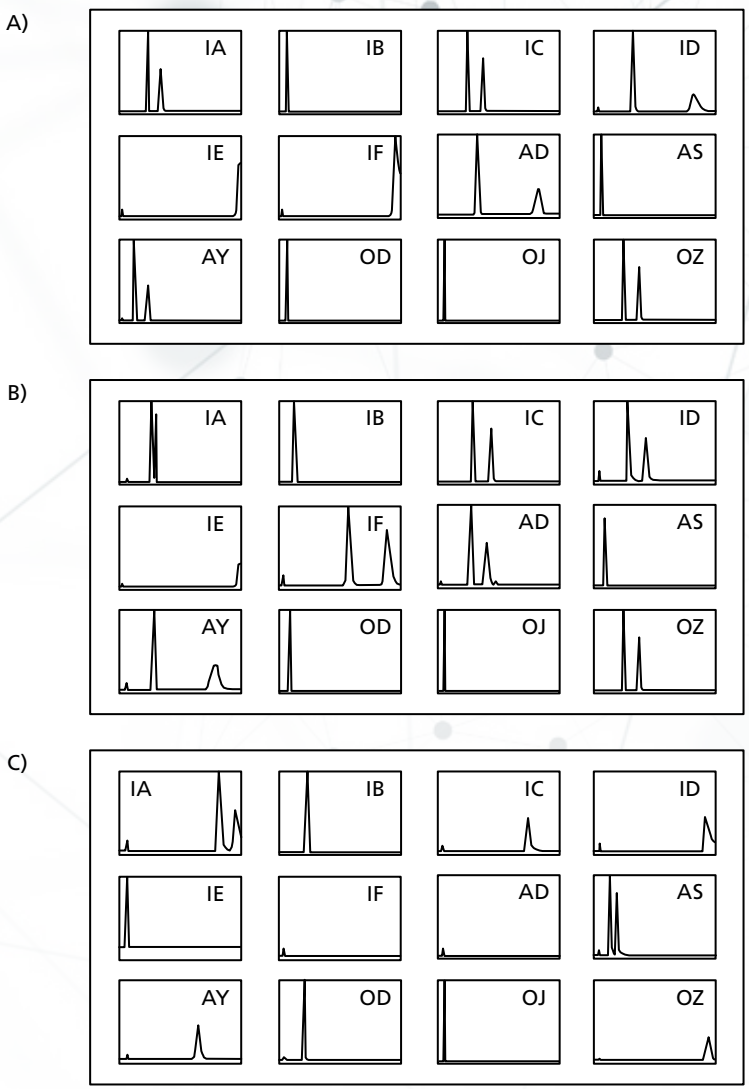
Method Scouting Solution Operating Window for Nexera UC/s

Ranking	Run No.	Analytical Condition	Resolution	Separation Factor	Symmetry Factor		Retention Factor		Area %		Peak Number
					Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2	
1	32	Omeprazole_OZ-3_MeOH_20_40	7.965	1.921	1.160	1.159	6.583	12.644	49.829	50.171	2
2	17	Omeprazole_IC-3_MeOH_20_40	5.587	1.602	1.387	1.274	8.078	12.937	49.971	50.029	2
3	16	Omeprazole_IC-3_EtOH_20_40	5.382	1.639	1.915	1.661	8.617	14.124	49.984	50.016	2
4	31	Omeprazole_OZ-3_EtOH_20_40	5.377	1.599	1.169	1.162	7.229	11.561	49.778	50.222	2
5	1	Omeprazole_AD-3_EtOH_20_40	3.996	1.509	1.257	1.404	8.779	13.250	50.054	49.946	2
6	8	Omeprazole_AY-3_MeOH_20_40	3.550	2.080	1.178	1.145	3.652	7.597	49.974	50.026	2
7	11	Omeprazole_IA-3_MeOH_20_40	3.428	1.523	1.464	1.312	7.435	11.327	49.973	50.027	2
8	4	Omeprazole_AS-3_EtOH_20_40	2.515	1.673	1.657	1.518	1.244	2.081	49.754	50.246	2
9	10	Omeprazole_IA-3_EtOH_20_40	1.586	1.157	1.322	1.279	7.115	8.234	49.347	50.653	2

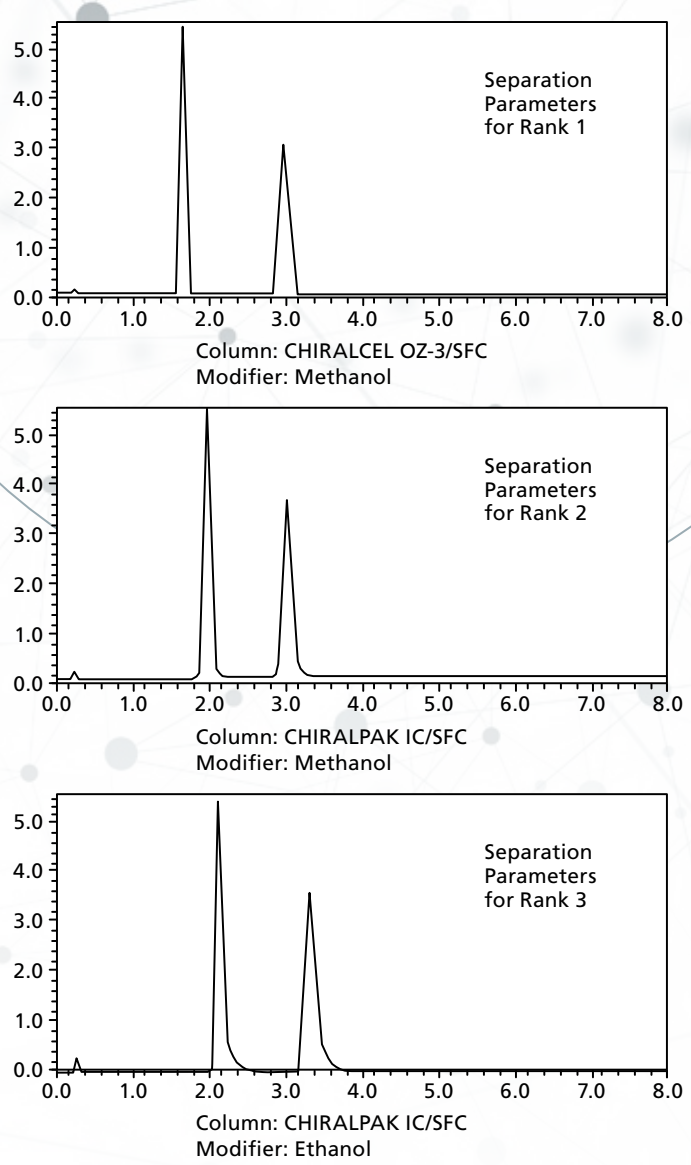
Using Software to Rank Separation Parameters

The table below lists the Daicel CHIRALPAK® and CHIRALCEL® chiral columns for SFC:

Chiral Columns Used for Separation Parameter Optimization	
Column	**Functional Group**
CHIRALPAK IA-3/SFC (IA)	Amylose tris (3, 5-dimethylphenylcarbamate)
CHIRALPAK IB-3/SFC (IB)	Cellulose tris (3, 5-dimethylphenylcarbamate)
CHIRALPAK IC-3/SFC (IC)	Cellulose tris (3, 5-dichlorophenylcarbamate)
CHIRALPAK ID-3/SFC (ID)	Amylose tris (3-chlorophenylcarbamate)
CHIRALPAK IE-3/SFC (IE)	Amylose tris (3, 5-dichlorophenylcarbamate)
CHIRALPAK IF-3/SFC (IF)	Amylose tris (3-chloro-4-methylphenylcarbamate)
CHIRALPAK AD-3/SFC (AD)	Amylose tris (3, 5-dimethylphenylcarbamate)
CHIRALPAK AS-3/SFC (AS)	Amylose tris [(S)-α-methylbenzylcarbamate)]
CHIRALPAK AY-3/SFC (AY)	Amylose tris (5-chloro-2-methylphenylcarbamate)
CHIRALCEL OD-3/SFC (OD)	Cellulose tris (3, 5-dimethylphenylcarbamate)
CHIRALCEL OJ-3/SFC (OJ)	Cellulose tris (4-methylbenzoate)
CHIRALCEL OZ-3/SFC (OZ)	Cellulose tris (3-chloro-4-methylphenylcarbamate)



Chiral Screening Separation Results.
Modifiers: (a) Methanol, (b) Ethanol, and (c) Acetonitrile/ethanol = 75/25 (v/v)



Chromatograms for Top Three Separation Parameters

The figure above (left) shows the results of separation patterns from a total of 36 possible combinations of 12 chiral columns and 3 types of modifiers (methanol, ethanol, and acetonitrile/ethanol mixture). Then optional software was used to rank the results, with chromatograms for the top three shown in the figure above (right). As a result, the parameters were successfully optimized while reducing the amount of work involved in the tedious process of optimizing separation parameters for chiral analysis by SFC.

The Nexera UC UHPLC/SFC system is a powerful, flexible tool for conducting analysis to evaluate drug components or separation conditions with simpler method development, a more efficient workflow and reliable, accurate results. Shimadzu complements the Nexera UC UHPLC/SFC system with an assortment of columns designed for high-resolution, high-speed separation of a wide variety of compounds using SFC.

To learn more, visit
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