USING SFC TO IMPROVE SEPARATION WORKFLOWS

FOR DRUG ANALYSIS AND PURIFICATION

SHIMADZU

Excellence in Science

Supercritical fluid chromatography (SFC) is a widely accepted technique for analytical and preparative separation of chiral and achiral compounds related to pharmaceutical development. Today's robust SFC instrumentation provides reliable, easy access to this extremely useful technique. While the user experience is enabled by thoughtful integration of analytical SFC, prep SFC, and a full range of SFC-MS options from single quadrupole to QTOF, all supported by a unified software platform.

This eBook examines chiral and achiral separation solutions using SFC for rapid screening of multiple analytical conditions, simplified method development, and purification at laboratory scale. The innovative engineering and task-focused workflows of the Nexera UC lineup are designed to take on the most challenging separations in drug development and purification.

Chiral Separation Using SFC for Pharmaceutical Analysis

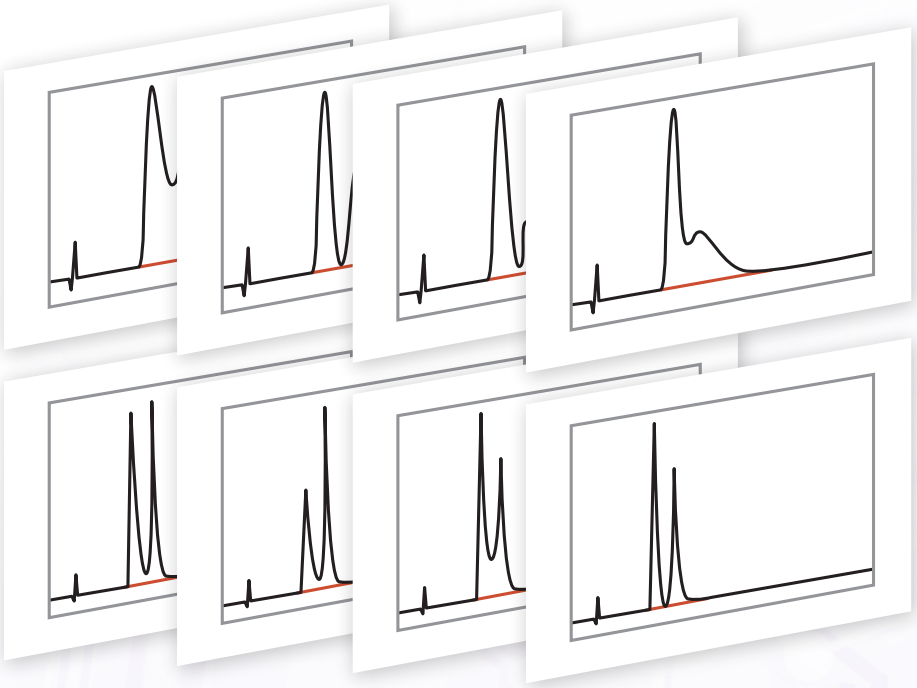
Normal phase HPLC has typically been used to separate chiral compounds, but now SFC has become a proven choice for chiral analysis and purification in the pharmaceutical industry because of its faster separations and analysis time, increased sensitivity and reduced solvent consumption.

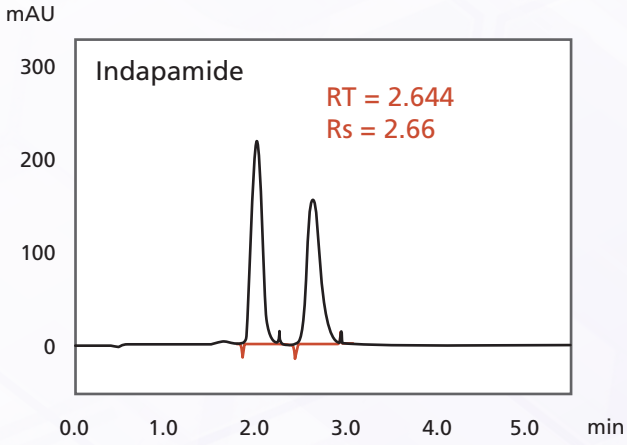
The main mobile phase used for chiral SFC analysis is supercritical carbon dioxide, with low polarity, low viscosity and high diffusivity. Polar organic modifiers, often alcohols, are added to control solubility and elution time. The use of additives in the modifier offers additional flexibility in method optimization.

To eliminate the time and effort required in chiral SFC to select various instrument parameters, such as columns and modifiers, the advanced SFC chiral screening system can automatically program the numerous method settings, while also accounting for necessary system and column equilibration when conditions are changed.

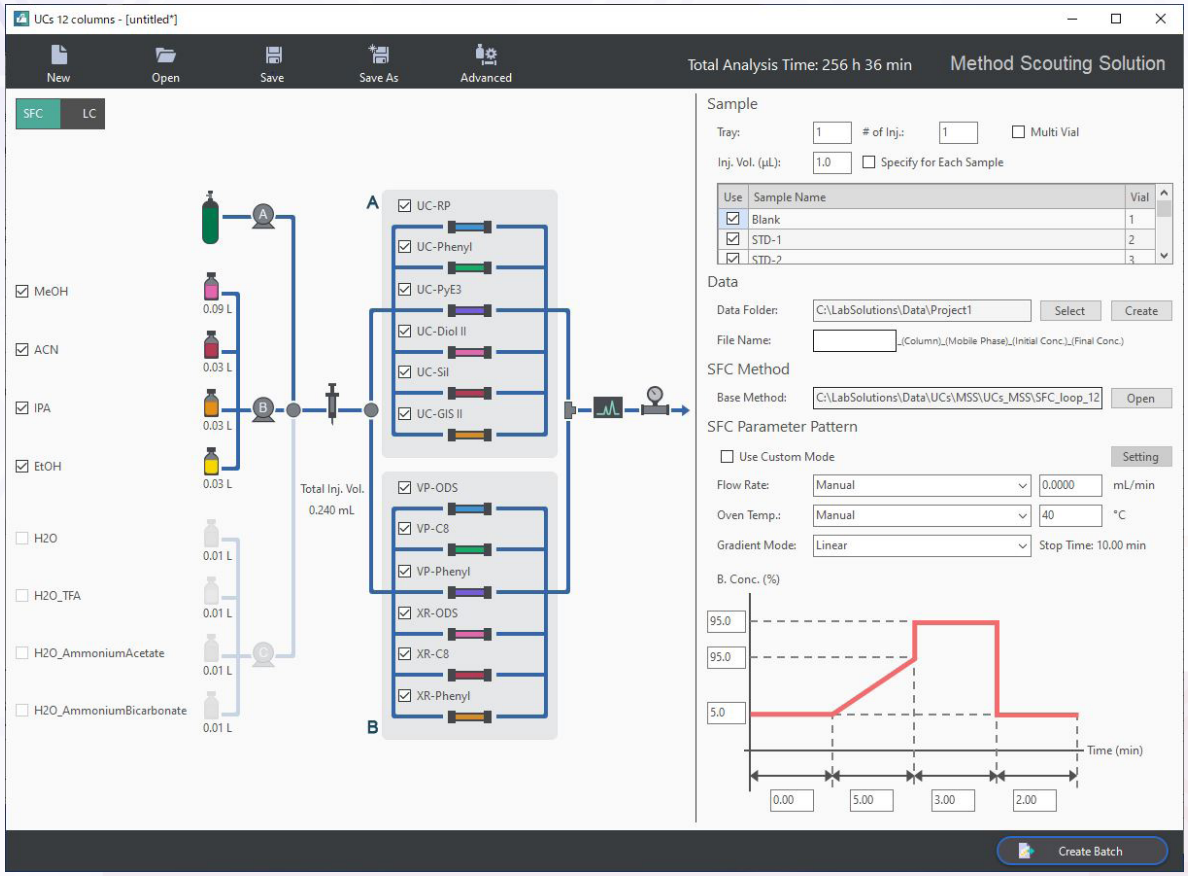
Evaluating Conditions for Chiral Analysis

With the Nexera UC Screening System, you can perform SFC analysis of chiral compounds in 1/3 to 1/5 the time compared to a conventional HPLC system. This screening system automatically switches between up to 12 columns, four modifiers, and blends of those modifiers. When configured with appropriate software, the creation of methods and batches can be automated, which eliminates tedious manual entry and avoids potential errors.





Columns: CHIRALPAK® AD-3
Modifier: Acetonitrile/Ethanol= 7/3



Usage can be tracked and exceptions can be created to prevent the use of incompatible solvents, excessive pressure, or restricted temperatures.

Method Scouting Solution

The Method Scouting Solution dedicated software enables analysts to manage columns and modifiers in a database and apply various analytical conditions by simply selecting them from the intuitive user interface. By organizing columns in the Method Scouting Solution database, usage can be tracked and exceptions can be created to prevent the use of incompatible solvents, excessive pressure, or restricted temperatures. Experiments can be designed to screen mobile phase and stationary phase selection, as well as testing gradient profile parameters, including complex multi-step gradients.

Nexera UC Switching System – Combining the Power of SFC and UHPLC

Harnessing the strength of modular design with the Method Scouting Solution platform, Shimadzu has developed a method screening system and workflow using both SFC and HPLC within a single instrument to evaluate chiral and achiral separations more efficiently. By adding an additional UHPLC pump to the Nexera UC Chiral Screening system, aqueous solvents can be added for use in UHPLC mode.

With a 12 column capacity, experiments can be run on 6 columns in SFC mode and 6 columns in LC mode in the same batch. Using this workflow, the operator can collect comprehensive data while automatically and continuously switching through multiple combinations of columns and mobile phases for both SFC and UHPLC analysis.

By adding an additional UHPLC pump to the Nexera UC Chiral Screening system, aqueous solvents can be added for use in UHPLC mode.

Nexera UC Switching System – Automated Mode Changes

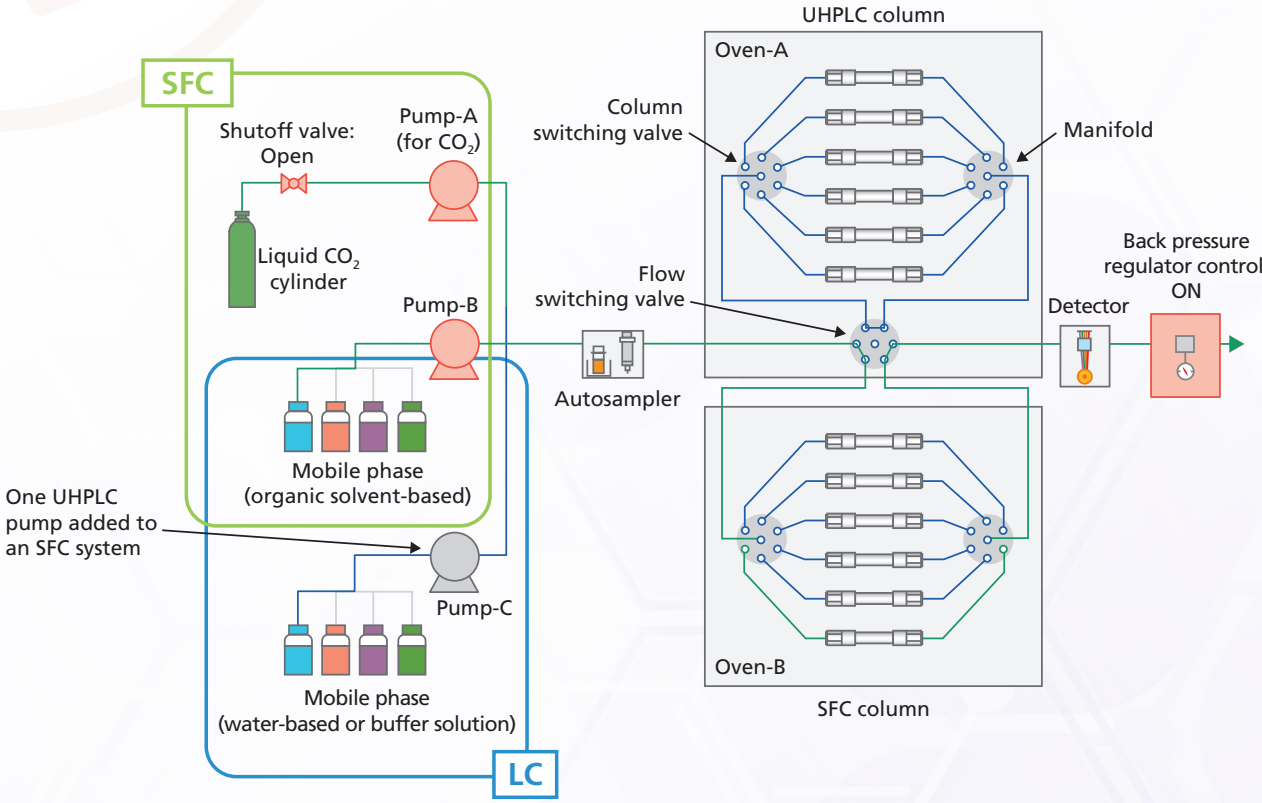
By coordinating the use of multiple pump and column valves, the Nexera UC Switching System can switch analytical conditions between SFC and UHPLC mode in approximately twenty minutes.

To switch from SFC to UHPLC, a solvent that is miscible with mobile phases in both modes (such as ethanol, isopropanol, or methanol) replaces the mobile phase containing CO_2 in the flow line.

Next, the second solvent delivery pump, typically carrying aqueous solvents, is enabled, and UHPLC mobile phase is delivered to the column for equilibration to the new mode. This is followed by equilibration to the next analysis method. The process of switching analytical conditions from UHPLC to SFC is virtually the same.

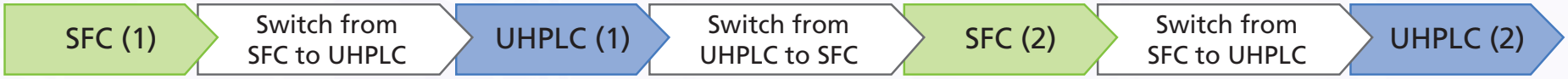
Cost and space requirements are minimized because both SFC and UHPLC share the solvent delivery unit for pumping organic solvents, autosampler, column oven, and detector. In addition, an existing UHPLC system can be upgraded to this system.

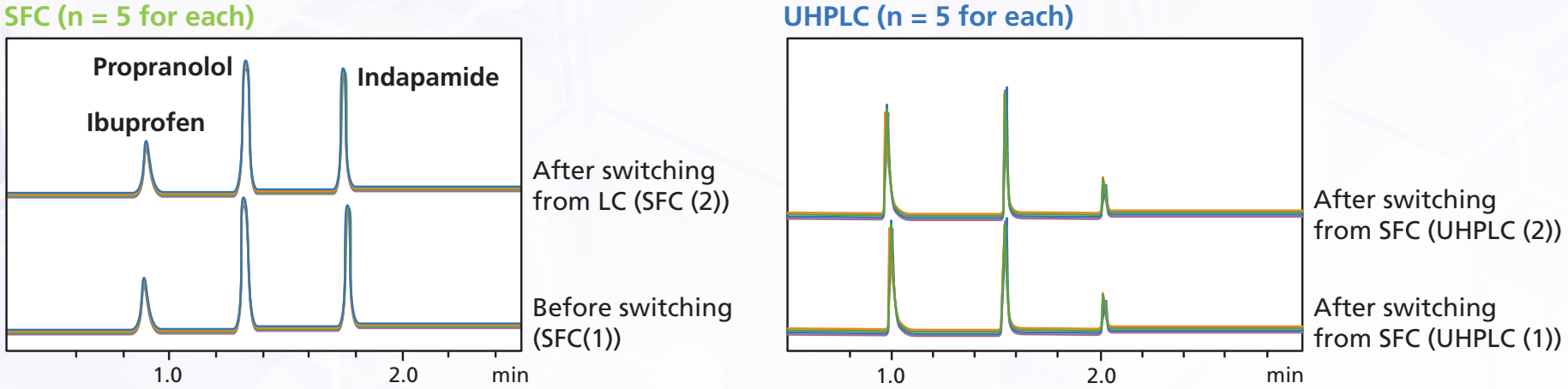
Nexera UC/s UHPLC/SFC switching system and flow line diagram (Equipment in green frame used for SFC)



Flow diagram for the Nexera UC SFC/UHPLC Switching System, which can be configured by adding a supercritical carbon dioxide delivery unit and back pressure regulator unit to a standard UHPLC system.

Chromatograms for three drug components, obtained by switching between UHPLC and SFC analysis modes





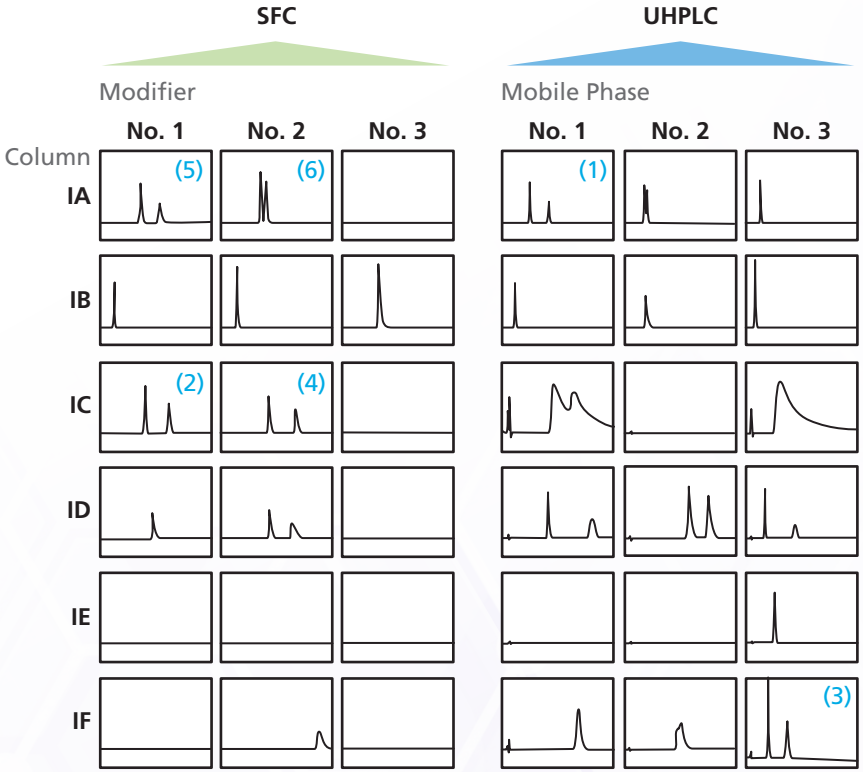
Reproducibility for UHPLC and SFC Switching

To demonstrate the reproducibility of UHPLC and SFC mode changes, we analyzed drug components propranolol, indapamide and ibuprofen. The resulting chromatograms shown above demonstrate reliable results, with no effects from switching flow lines three times, even though mobile phases and separation characteristics were significantly different.

Faster Scouting for Chiral Method Development

Here is an example of using the Nexera UC Switching System to quickly screen separation conditions for the racemic drug omeprazole.

We evaluated 6 CHIRALPAK® columns against 3 mobile phases in SFC and UHPLC modes. The resulting 18 combinations each for UHPLC and SFC can be quickly visualized. Ranking the results for peak resolution and peak shape, we see that both normal phase and SFC conditions can produce excellent separation. When factoring in solvent purchase and disposal costs, as well as exposure risks, the move from normal phase conditions to SFC is both ecologically and economically sound.



Chromatograms of omeprazole under 36 different analytical conditions

Table 1: UHPLC Analytical Conditions for Chiral Compounds

No.	Mobile phase (Upper: A and Lower B)	Otherv
1	Hexane Ethanol	**B Conc. (%):** 20% (Isocratic) **Flow Rate:** 2 mL/min **Column Temp:** 40°C **Inj. Volume:** 1 µL **Detection:** PDA @ 220 nm **Step GE** 0 – 6 min 20% Analysis 6 – 8 min 40% Column washing 8 – 12 min 20% Equilibration
2	Hexane Isopropyl Alcohol	
3	Methyl Tertiary Butyl Etherol	

Table 2: SFC Analytical Conditions for Chiral Compounds

No.	Mobile phase (Upper: A and Lower B)	Otherv
1	Hexane Ethanol	**Modifier Conc. (%):** 20% (Isocratic) **Flow Rate:** 3 mL/min **Column Temp:** 40°C **Inj. Volume:** 1 µL **BPR Press:** 10 MPa **Detection:** PDA @ 220 nm **Step GE** 0 – 5 min 20% Analysis 5 – 7 min 40% Column washing 7 – 10 min 20% Equilibration
2	Hexane	
3	Acetonitrile / Ethanol = 75 / 25 (v/v)	

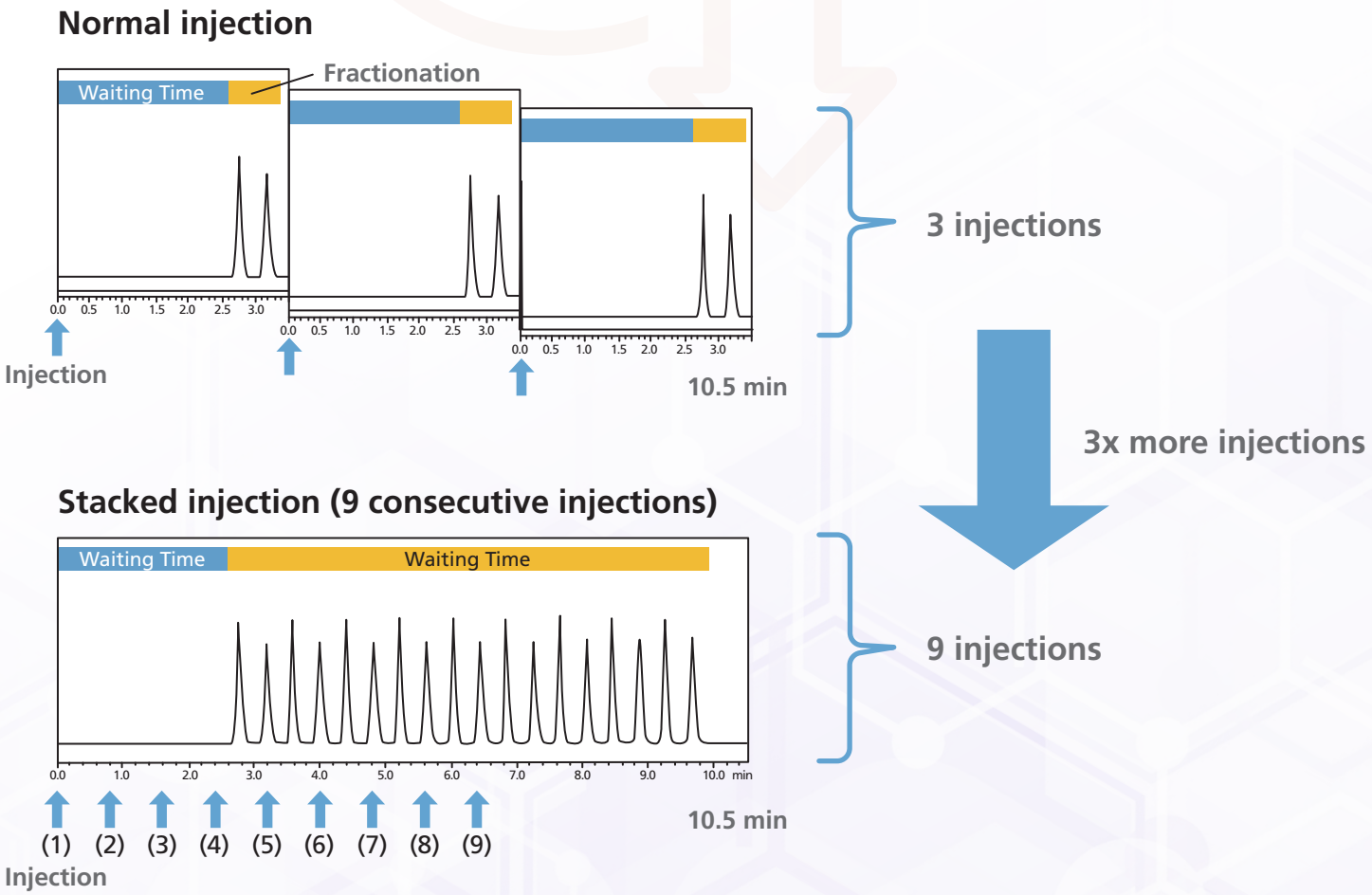
Semi Preparative SFC System

With a foundation of shared technology like the revolutionary piezo-controlled two-stage back pressure regulator (BPR), the Nexera UC Prep system offers both the high-performance achieved in the analytical Nexera UC model and purpose-built preparative SFC technologies.

In preparative SFC, recovery of the separated fraction depends on the desired compound remaining in the solution, while minimizing both losses to dispersed sample and total collected volume. As CO_2 rapidly expands after the BPR, many gas liquid separation schemes, like cyclonic separation, allow dispersed sample to be carried away as an aerosol, or deposited in the separation device, leading to low recovery and potential sources of carryover. The Nexera UC Prep's patented LotusStream gas-liquid separator reduces sample dispersion and carryover, while achieving high recovery rates. The use of the Coanda effect in designing the LotusStream allows even small amounts of modifier and makeup flow to be used to recover high yield fractions without precipitation. Even semi-volatile compounds that are subject to loss in cyclonic separation, such as linalool and limonene, are recovered at higher rates with the LotusStream technology.

Semi Preparative SFC System – Stacked Injection

Operators can continuously inject samples with the Nexera UC Prep Stacked Injection system, which saves time and enables the processing of large amounts of material for purification. The figure below shows the chiral separation of warfarin via normal injection and stacked injection.



Chiral Separation of Warfarin

Semi Preparative SFC System – Multi-Fraction System

For workflows that require a greater number of fractions per run, or where a variety of smaller scale purifications are desired, the Multi-Fraction System is ideal. With the ability to inject 2 mL on column per cycle, the system excels at tasks such as impurity isolation, metabolite isolation, and other <100 mg purification needs. Utilizing a flexible open-bed fraction collector with a single LotusStream gas liquid separator, collection format options range from 96 well plates, test tubes from 11-35 mm, as well as custom tray teaching.

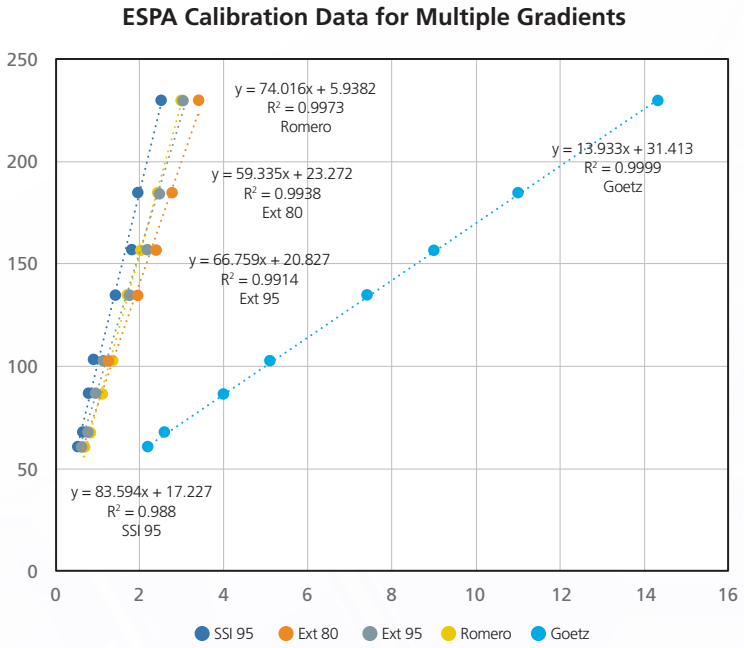
Achiral Separation Using SFC for Pharmaceutical Analysis

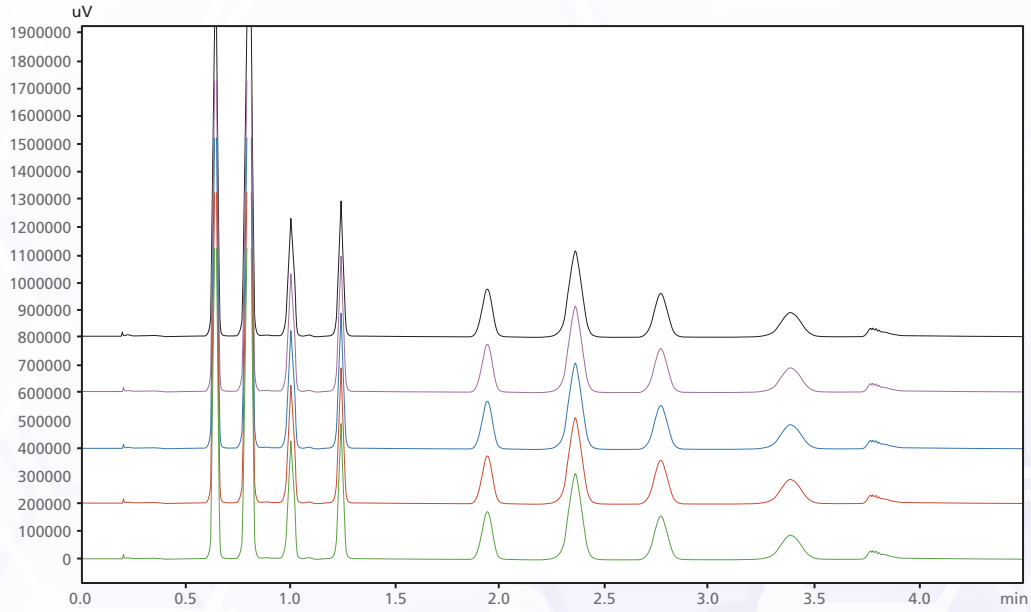
SFC has become the technique of choice for preparative chiral separations due to its unique selectivity for chiral resolution compared to HPLC. Today, SFC sample preparation and analysis have expanded to achiral separations to support drug discovery and development thanks to advanced analytical and purification SFC instruments, fast separation speed, lower solvent consumption and availability of a wide range of SFC stationary phases.

When coupled with mass spectrometry, SFC-MS has been shown to provide compound-dependent gains in sensitivity, and has started to find a role in bioanalysis, ADME, and DMPK for that reason.

Emerging applications like Exposed Polar Surface Area (EPSA) assays are bringing SFC-based techniques to the field of cyclic peptides, allowing a sub-10 minute SFC run to mimic the results of traditional cell uptake studies.

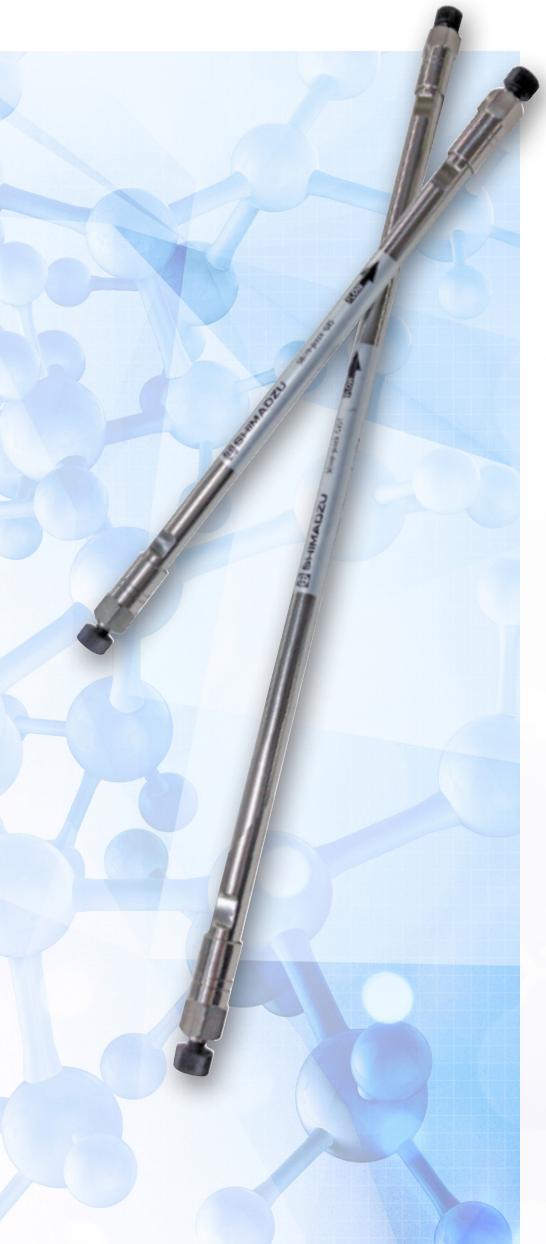
Techniques that avoid the supercritical regime, but use liquid CO_2 as a solvent, such as Enhanced Fluidity Liquid Chromatography, are expanding the technique into use with polypeptides and oligonucleotides. The flexible configuration options and performance characteristics of the Nexera UC series are ready for these new SFC spaces and the challenges that are to come.





Nexera UC Columns for SFC

The Nexera UC series columns were designed specifically for use with SFC and include fourteen achiral types of stationary phases with a range of sizes to meet diverse research and development needs. Columns are available in Sub-2-, 3- and 5-micron particle sizes. Analytical and preparative scale available.



Nexera UC SFC achiral separation columns include:

- Amine columns with a high-density NH2 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

The Nexera UC SFC system is a powerful, flexible tool for conducting chiral and achiral analysis enabling researchers to rapidly screen multiple SFC analysis conditions, access emerging separation techniques, and collect high-purity, high-yield fractions in purification.

To learn more, visit
www.MustSeeSFC.com

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