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# FREQUENTLY ASKED QUESTIONS
## Supercritical Fluid Chromatography

### 1 Why is carbon dioxide used for supercritical fluid chromatography (SFC)?

While many potential solvents, such as water and ethane, can be enhanced by use in a supercritical fluid state, $CO_2$ stands out as an ideal choice for chromatography applications. With critical points at 31 °C and 10 MPa, $CO_2$ becomes a supercritical fluid under modest conditions, especially compared to the 373 °C and 22 MPa observed for water. $CO_2$ is not flammable and has minimal risk of explosion even if used in a compressed state. In addition, it offers intrinsically low toxicity when compared to other organic solvents. From a cost perspective, it is mainly sold as a liquid stored in high-pressure cylinders and in most cases can be acquired at a low cost. In addition, liquified carbon dioxide gas is collected as a byproduct of fermentation processes or chemical plants. That means using liquified $CO_2$ causes minimal environmental impact.

### 2 What advantages does SFC offer over HPLC?

Supercritical carbon dioxide offers the low polarity of normal phase solvents like n-hexane, while still being miscible with methanol, which is a water-soluble solvent. Additionally, supercritical carbon dioxide has lower viscosity than solvents such as water, which results in lower column back pressure for equivalent conditions, allowing the potential to speed up analysis without over-pressuring the system. Coupling this advantage with the higher linear velocities of SFC for optimal theoretical plate height can potentially result in faster and better separations. Because SFC consumes less organic solvent and involves treating less liquid waste, the overall solvent cost including disposal is lower than for HPLC.

### 3 What are the key hardware differences between an HPLC and an SFC? Will these differences require a separate setup/system?

There are four major differences between SFC and HPLC configurations.

1. Pumps Designed Specifically for Supercritical Carbon Dioxide – The delivery pump must have built-in cooling functionality to maintain the liquid state as pressure exerted on the solvent is increased. Heat created by the pumping process is removed by coolant circulating through the pump heads.

2. Back Pressure Regulator (BPR) – A unit is required to keep the $CO_2$ in a solvent state (liquid or supercritical fluid) within the system and prevent it from vaporizing by maintaining the pressure level within flow channels. In an SFC system, the BPR is positioned downstream from an ultraviolet (UV) or photodiode array (PDA) detector, or upstream from an evaporative light scattering detector (ELSD) or mass spectrometer (MS). The BPR detects pressure in the flow channels and then rapidly opens and closes to maintain a constant pressure within the flow channels.

3. Modifier Delivery Pump – For SFC analysis, an organic solvent such as methanol or acetonitrile (modifier) is pumped for mixing with the supercritical carbon dioxide. That means separate pumps are required for pumping the supercritical carbon dioxide and modifier. A standard UHPLC/HPLC pump is used for this duty in SFC.

4. Make-up Delivery Pump – If an ELSD or MS is used for detection or if the system is used for preparative separation, then a solvent (make-up solvent) is pumped to prevent precipitation in the flow channels or to improve the recovery rate of components in separated fractions. Make-up solvent is also pumped to improve sensitivity and promote ionization during MS detection.

With the addition of a third solvent delivery pump, it is possible to combine SFC and UHPLC operation into a single system. Along with user-friendly software, this hybrid system can enable even new users to configure experiments for both separation modes in a single batch.

## 4 Is it possible to use both HPLC and SFC for method development? If so, what steps need to be taken?

Shimadzu offers true flexibility in chromatography method development, with a hybrid UHPLC/SFC system, providing the power of both techniques in a single instrument. Using a three-pump solvent delivery system combined with a dedicated method development user interface, use in UHPLC or SFC mode is automatically managed, including system solvent flushing and column equilibration. Whether SFC will be 90% or 40% of projected workflow, system changeover and the ability to provide high performance in either mode is quickly and easily accessible.

## 5 About how much $CO_2$ gas is used per analysis?

Assuming ten minutes per analysis and pumping 100% $CO_2$ at a flowrate of 3 mL/min, about 30 g is used per analysis. Use that value to recalculate usage based on appropriate changes to the analysis time, modifier ratio (which reduces the proportion of $CO_2$) and flowrate.

Typical CGA 320 – 200 cylinders contain about 20 kg of useable liquid $CO_2$, or over 100 hours of run time at 3 mL/min.

## 6 While I'm interested in SFC, are there certain samples better suited to HPLC analysis?

Samples that will dissolve only in highly aqueous conditions may not be suitable for SFC analysis. As a general rule, any sample that is soluble in methanol can be run under SFC conditions. Emerging techniques using SFC hardware and solvent systems do incorporate water as a co-solvent, usually at concentrations of less than 3%, and in combination with a water-miscible modifier, like an alcohol. Biologically relevant molecules such as polypeptides and oligonucleotides are the subject of ongoing SFC research.

## 7 What are the primary applications for SFC? Will this range of applications expand?

SFC has traditionally been used for chiral and achiral small molecule applications, with a focus on purification tasks. As modern analytical SFC systems are increasingly paired with mass spectrometry detection, applications such as pesticide and herbicide testing, bioanalysis (including chiral metabolite analysis) and exogenous chemical exposure testing are being adapted to SFC separation. Pharmaceutically relevant analyses, like Exposed Polar Surface Area (EPSA), have become important preclinical development tools, allowing rapid chromatographic methods to screen cell uptake potential of cyclic-peptide drug candidates.

## 8 Is it possible to use preparative SFC for purification? If so, does it offer any advantages?

Preparative separation by SFC offers a few big advantages. Supercritical carbon dioxide evaporates at ambient temperature and pressure conditions, which greatly reduces dry-down time and input energy of collected

fractions. Compared to reverse phase (RP) preparative HPLC, SFC fractions typically contain little or no water and have greatly reduced total volume. The solvent cost for SFC preparative separation is lower due to the use of inexpensive and environmentally friendly $CO_2$, as well as the lower concentrations of organic modifier needed for separation, which are typically 30% or less of total flow. While acetonitrile can be used as an organic modifier for SFC, so can alcohols, which could provide additional cost savings compared to RP preparative HPLC.

## 9 How can the expansion of $CO_2$ during the transition from the supercritical fluid state to the gas state in preparative SFC be controlled to avoid spatter, aerosol formation and other unwanted events that reduce fraction recovery?

The newly developed LotusStream (patented) gas-liquid separator splits incoming flow into multiple channels with the same inner diameter, rapidly decreasing linear velocity without inducing eruptive expansion. This technology enables the liquid portion to coalesce along the surface of the LotusStream, while the $CO_2$ is vented. As a result, the $CO_2$ is discharged externally, and the liquid travels along the separator and drips directly into the fraction vessel without spattering. Even at 150 mL/min of flow, liquid drips as a controlled stream directly to the vessel.

## 10 When screening columns for SFC, what separation parameters should be considered?

SFC mobile phases can accommodate a wide range of column types. Nearly all stationary phases that are used for normal phase, reverse phase and chiral separations can be used under SFC conditions. Additionally, the ability to change the backpressure setting can have an effect on separation. To screen for the best combination of mobile phase and stationary phases, mobile phase conditions are fixed and tested against a variety of columns. Automation of this task, with a method scouting system, automatically sets and executes respective parameter combinations, eliminating tedious and error-prone steps of experimental design to ensure reliable results.

## 11 We have been struggling with matrix effect problems in our LC/MS analysis. Will the same phenomena occur for SFC as well?

Ionization can be inhibited by the matrix even for SFC/MS. However, because LC and SFC can result in significantly different separation behavior, SFC/MS can often resolve such LC/MS matrix effect problems. Retention of polar compounds that often elute early under reverse phase conditions can be radically altered with SFC chemistry, offering selective control with modifier concentration.

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SHIMADZU
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800.477.1227 / 410.381.1227
www.ssi.shimadzu.com

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