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Rewriting the Book on Supercritical Fluid Chromatography

Fluids become supercritical when they are highly compressed and take on the properties of both a liquid and a gas. Supercritical carbon dioxide (CO_2) can be used as the mobile phase in supercritical fluid chromatography (SFC).

Until recently, this technique had been overlooked in favor of high-performance liquid chromatography (HPLC) and gas chromatography (GC). However, new developments in the field have demonstrated that SFC can be a highly cost-effective, accurate and sensitive solution capable of outperforming HPLC and GC. In light of these new technologies, it's time to reshape the field of chromatography with SFC.

Back to basics: SFC and supercritical CO_2

A supercritical state occurs when a gas or liquid is subjected to temperatures and pressures that exceed its critical point.

In this state, it will display properties of both liquids and gases, the advantages of which can be applied to SFC:

Diffusivity comparable to a gas	Increased density comparable to a liquid	Dynamic viscosity comparable to a gas
Improved solvent capabilities	Rapid carrying and eluting for an efficient mobile phase	High flow rate and variable solvation strength

Using supercritical fluids for chromatography is not a novel idea. In fact, supercritical fluids have been used as a mobile phase for separation for over 60 years, emerging originally under the name "high-pressure gas chromatography".

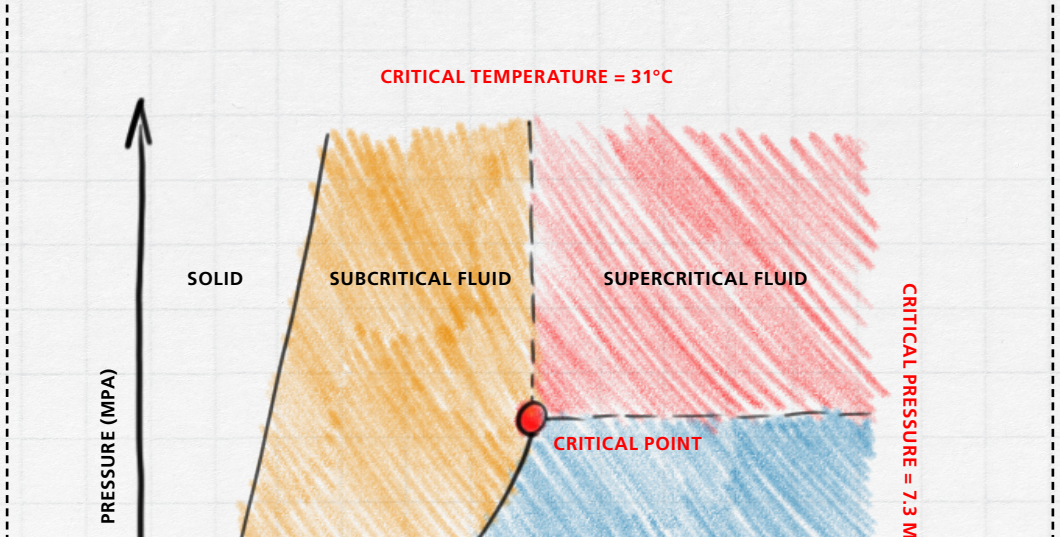
HPGC was initially performed using supercritical monochloro-difluoromethane and dichlorofluoromethane, before carbon dioxide (CO_2) was discovered as a potential mobile phase.[1,2]

Supercritical extraction and chromatography has been used in industrial applications for decades, beginning with the decaffeination of coffee in the 1970s.[3]

Why CO_2?

CO_2 has several advantages over other supercritical fluids, making it the standard mobile phase for high-performance SFC.

CO_2 Phase Diagram



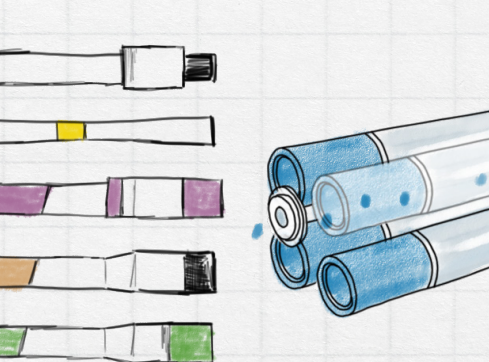
Advantages of CO_2

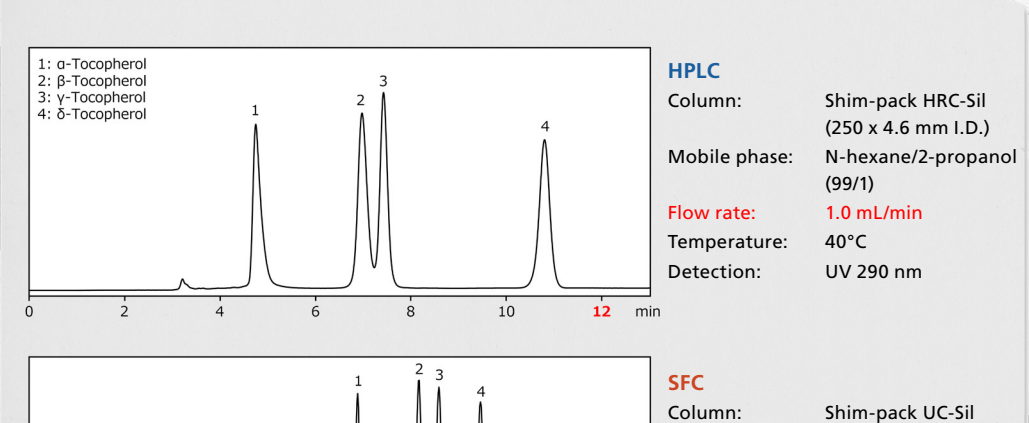
- Critical temperature and pressure can be achieved without environmental extremes
- Chemically inert
- Non-toxic and non-corrosive
- Inexpensive and abundant at high purity
- Miscible with most co-solvents, allowing dissolution of a wide range of both polar and non-polar analytes
- Reverts to a gas at room temperature and atmospheric pressure, resulting in high recovery rates and rapid purifications

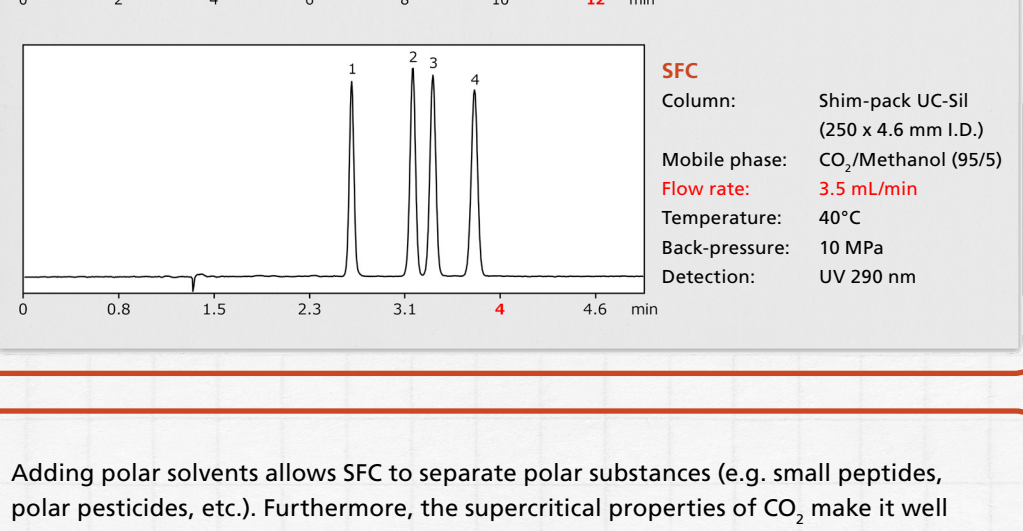
Time to rethink supercritical fluid chromatography

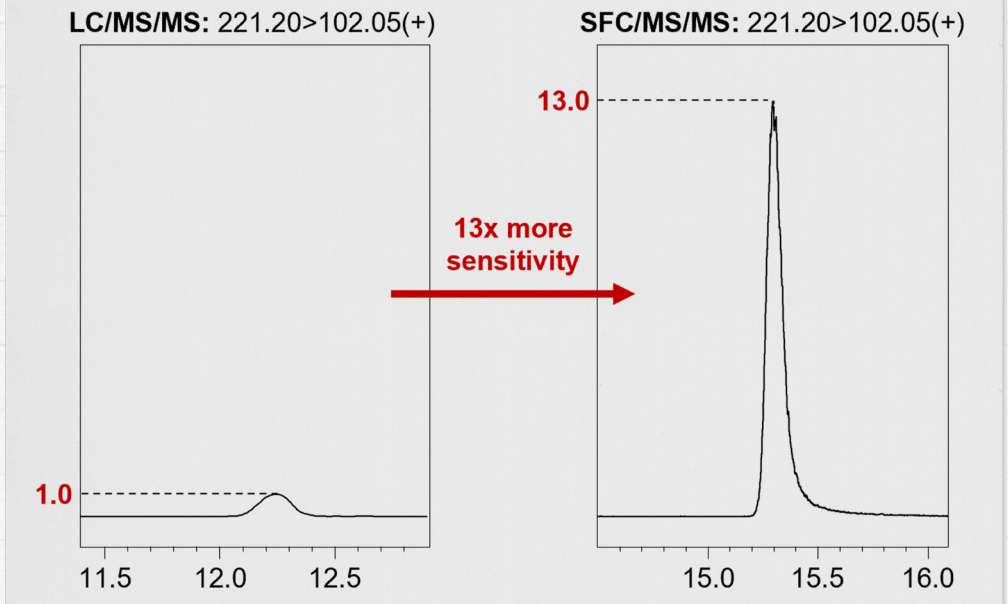
Over its lifetime, SFC has often been overshadowed by other chromatography techniques. As a result, many first-time users of SFC may have misconceptions about the modern face of this technique, but it's time to set the record straight.

Misconception	Fact
SFC is only useful for chiral molecules	SFC can be used to separate both chiral and achiral molecules and is often used to separate pharmaceutically active compounds such as vitamins, steroids and barbiturates.
SFC requires specialized expertise and equipment	The same principles of chromatography apply to both HPLC and SFC, meaning that expertise is fully transferable. Additionally, the columns (e.g., silica and octadecyl) and detectors (e.g., photodiode array (PDA) and mass spectrometry) used in HPLC can be directly transferred to SFC.
SFC is expensive and time-consuming	SFC systems have a higher benefit-to-cost ratio compared to HPLC, as the low viscosity and high diffusivity of supercritical CO_2 allow for higher flow rates for faster analysis and separation, with no loss of sensitivity.
CO_2-based SFC can only be used for non-polar compounds	Adding polar solvents allows SFC to separate polar substances (e.g. small peptides, polar pesticides, etc.). Furthermore, the supercritical properties of CO_2 make it well suited to comprehensively analyze compounds over a wide polarity range not possible with HPLC or GC alone.
Detection isn't sensitive enough for many applications	Coupling SFC with a mass spectrometer (MS) can enable greater sensitivity in some circumstances than LC/MS. This is due to an alternative ionization mechanism caused by the interaction of CO_2 with the solvent.









Suitable for a wide range of applications

SFC is a well-established technique in the pharmaceutical industry. However, it is also suitable for a wide variety of industries and applications:

PHARMACEUTICS
- Purification of drugs
- Analysis of drug formulations
- Chiral separations

BIOANALYSIS
- Pharmacokinetic studies
- Forensic applications
- Lipidomics

FOOD SCIENCE
- Contaminant analysis
- Quality control
- Analysis of complex compounds, e.g., lipids, pesticides

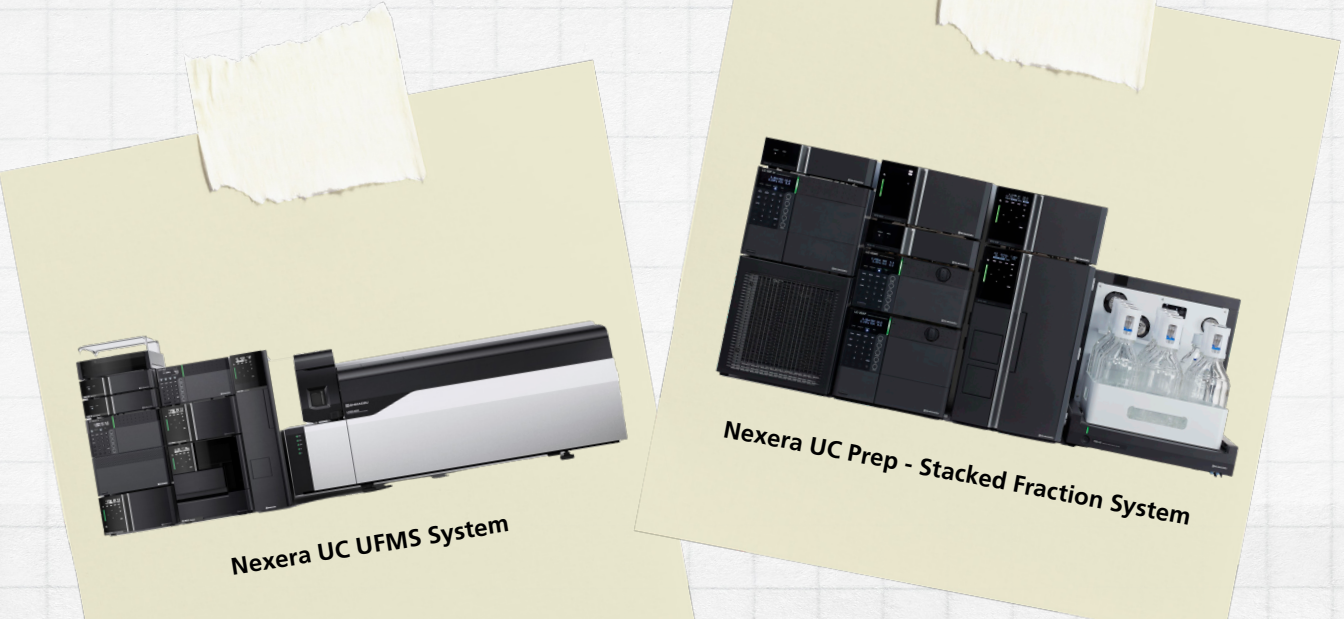
NATURAL PRODUCTS
- Bioactivity analysis in traditional medicine
- Quality control and contaminant analysis
- Production of sustainable natural products

End-to-end solutions with Shimadzu

Shimadzu has created a comprehensive "Unified Chromatography" analytical scale system, in Nexera™ UC; but what is Unified Chromatography? UC characterizes the use of a very wide gradient, from a CO_2-rich supercritical/ sub-critical mobile phase (SFC) to a completely liquid mobile phase like in LC.[1] This is a versatile technique that allows separation of hydrophobic and hydrophilic compounds in a single analysis. To meet current needs for a reliable preparative scale instrument, Shimadzu has worked closely with the Enabling Technologies Consortium (ETC), a group of pharmaceutical and biotechnology companies, and released the Nexera™ UC Prep. This system offers the high performance of the Nexera UC with reliable high-performance semi-prep purification. Both systems also enable the on-line extraction (SFE) and analysis (SFC) of a wide range of target samples for high-sensitivity detection and/ or purification.

HIGHLY VERSATILE CHROMATOGRAPHY: NEXERA UC AND NEXERA UC PREP

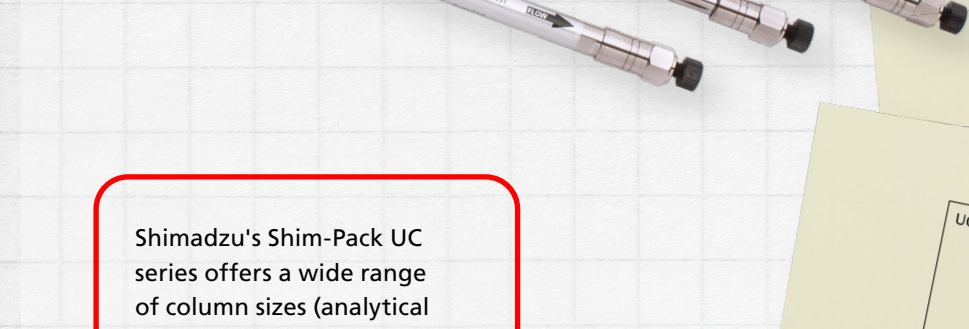
- Analytical scale UHPLC/SFC switching system allows screening by UHPLC and SFC in same batch
- Fractionation/purification at both analytical and preparative scales with addition of a fraction collector
- 94.2% reduction in organic solvent consumption for improved sustainability
- High resolution even at high flow rates
- Fully automated on-line extraction and separation

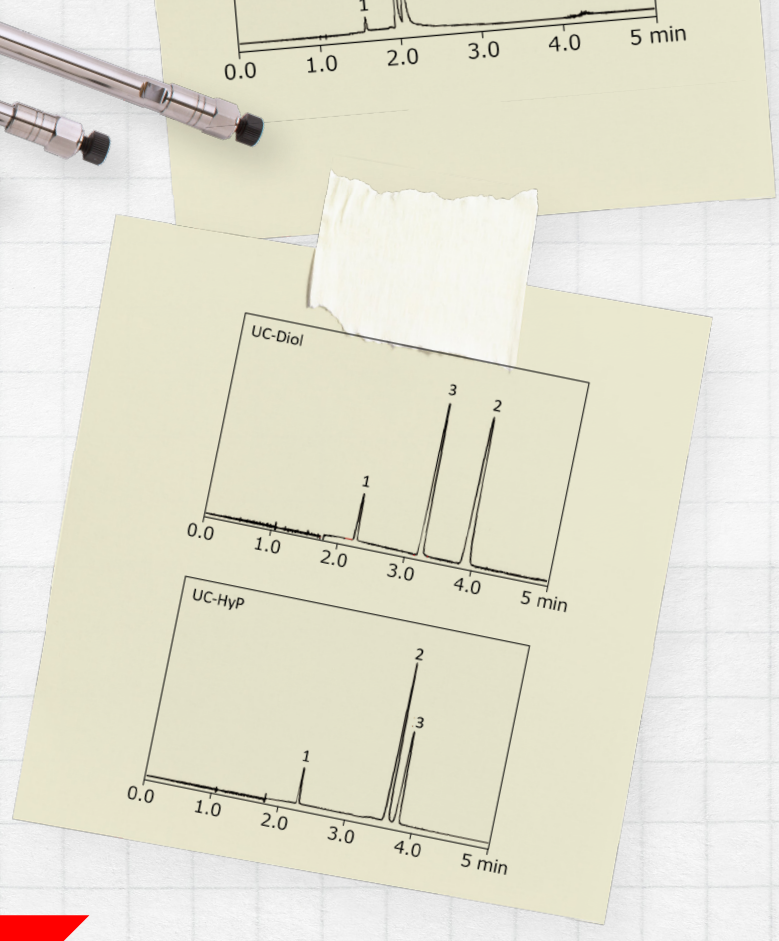


Meeting every need: Shim-pack UC columns

Due to the gas-like properties of the SFC mobile phase, diffusion of the sample band is higher than in liquid chromatography. Although standard HPLC columns can be used for SFC, higher speed and performance can be achieved with dedicated columns.

Separation of the same three analytes in different columns shows that retention behavior differs significantly according to the column chosen.



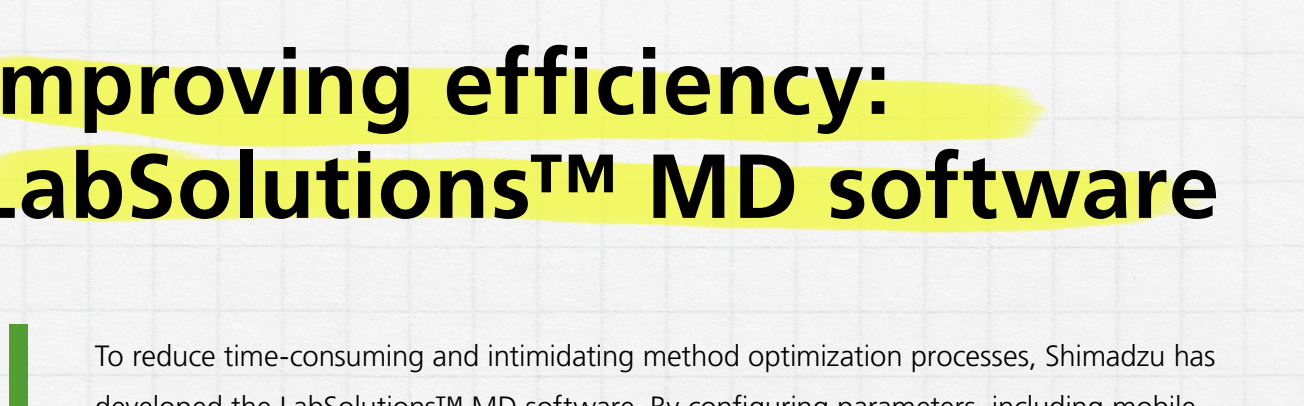


Shimadzu's Shim-Pack UC series offers a wide range of column sizes (analytical and preparative scale), and packing materials, to ensure a solution for almost every analyte.

Improving efficiency: LabSolutions™ MD software

To reduce time-consuming and intimidating method optimization processes, Shimadzu has developed the LabSolutions™ MD software. By configuring parameters, including mobile phases and columns, analysis schedules are automatically generated, and optimal analytical conditions are determined. This allows for simpler, more efficient and beginner-friendly experimental design.

AQbD methods can significantly reduce the number of data points needed for optimization – by up to 52%



Make analysis schedule based on experimental design → Analysis with automated switching of co-solvents, columns and SFC parameters → Building of Design Space with chromatogram simulation

Transform your chromatography with SFC

SELECT COLUMNS | OPTIMIZE YOUR WORKFLOW

References

1. Gros Q, Duval J, West C, Lesellier E. On-line supercritical fluid extraction-supercritical fluid chromatography (SFE-SFC) at a glance: A coupling story. *TrAC Trends in Analytical Chemistry.* 2021;144:116433. doi:10.1016/j.trac.2021.116433
2. Communications to the editor. *The Journal of Organic Chemistry.* 1962;27(2):700-706. doi:10.1021/jo01049a069
3. Zosel K; Studiengesellschaft Kohle mbH. Process for the decaffeination of coffee. United States patent 4260639A. 1970.